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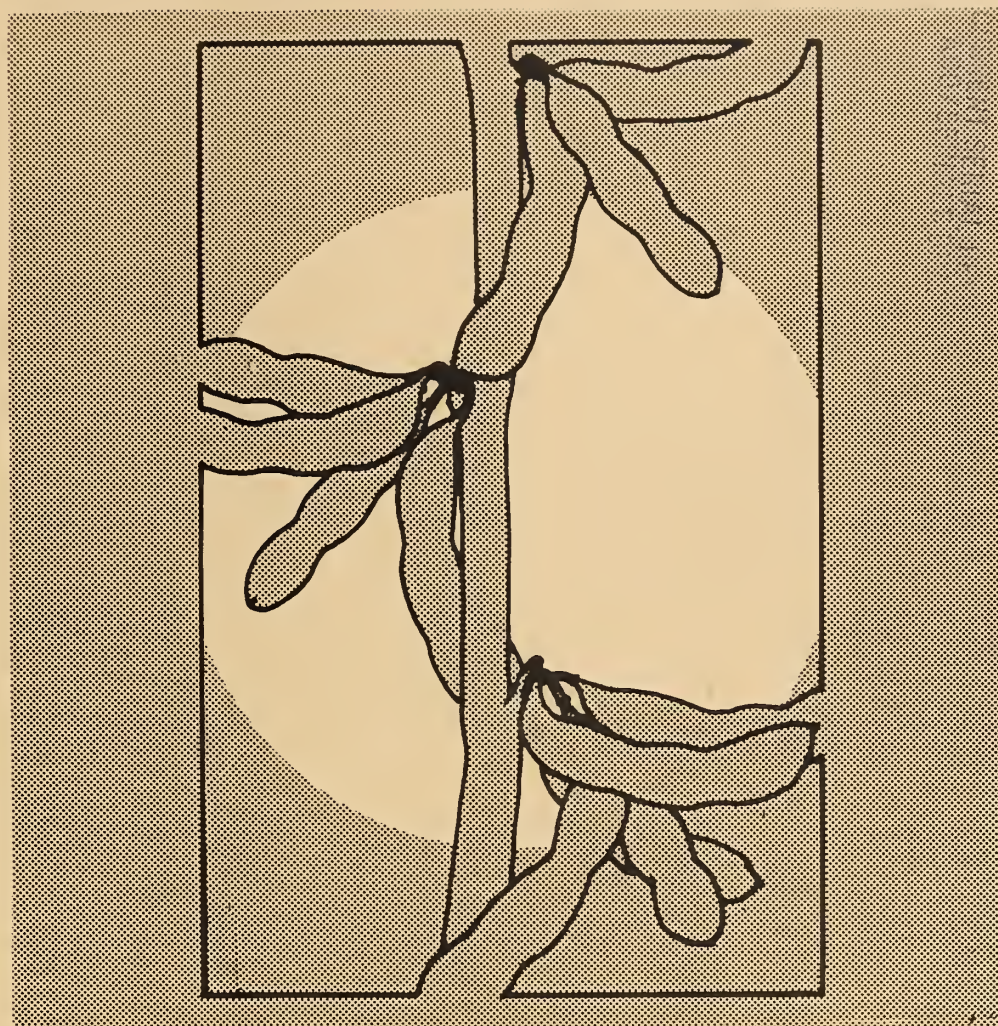
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Soybean Genetics Newsletter



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Volume 18

April 1991

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Agricultural Research Service - USDA
Department of Agronomy
and Department of Genetics
Iowa State University
Ames, Iowa 50011

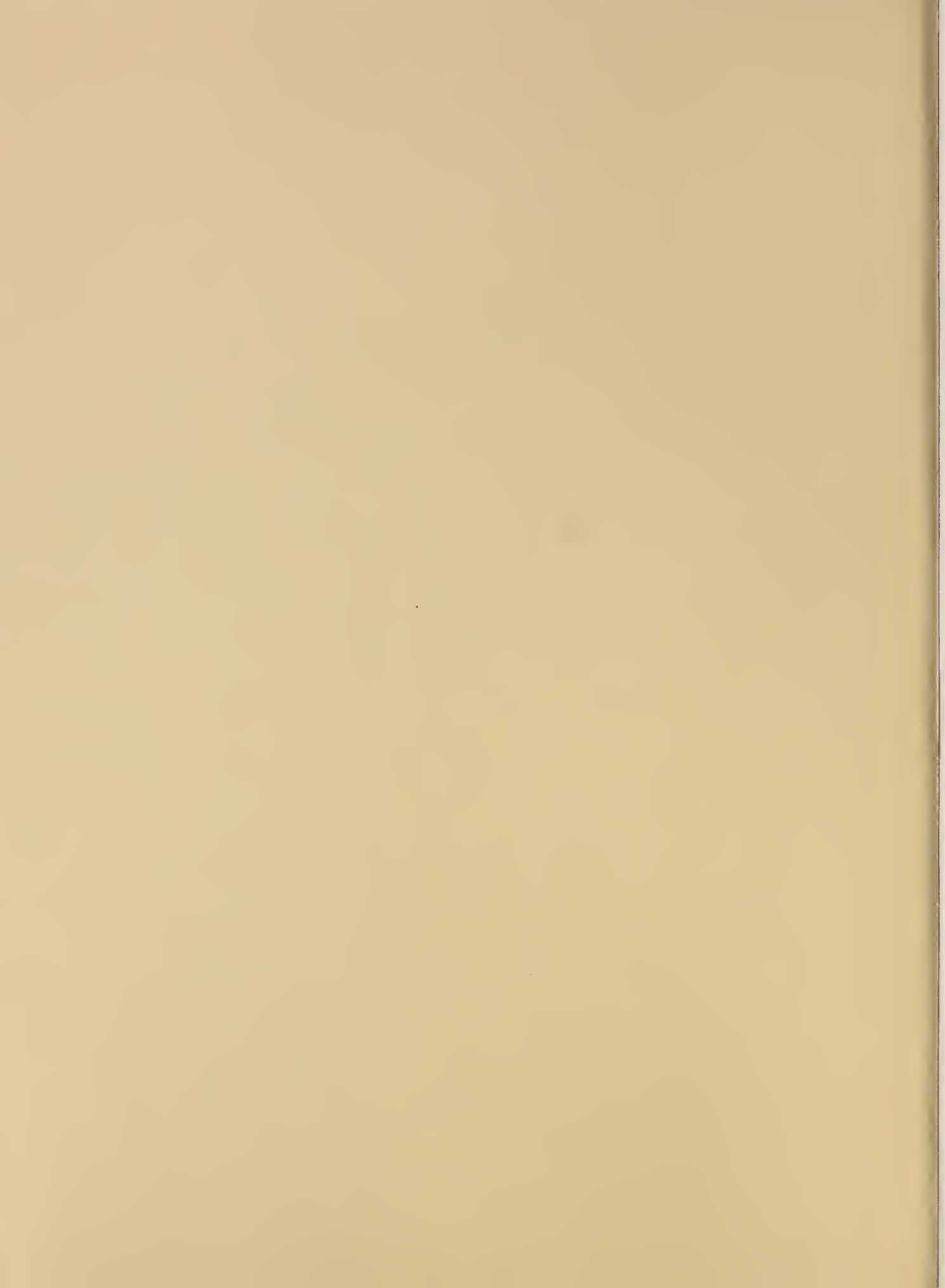


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FOREWORD

In 1990, when we found it necessary to institute a subscription fee for the Soybean Genetics Newsletter, we were naturally apprehensive concerning the response of readers and potential authors of news notes and articles. As you can see by the quantity and quality of Volume 18--this year's Newsletter--our worries were unnecessary.

The response of soybean scientists all over the world to the new Newsletter has been most gratifying to those of us responsible for publishing it. The worldwide derivation of the articles, from several continents and many nations, and the broad scope of themes--from breeding and pest resistance to chemical constitution and gene transfer--confirms our original belief, stated in Volume I, 1974, that soybean researchers the world over needed a forum ... "of an informal nature to stimulate thought and exchange ideas.... preliminary in nature and speculative in content. ... Even so, such reports can be exceedingly valuable and helpful, if viewed in the proper perspective."

The response of individuals, universities and institutions has been most gratifying. Especially appreciated is the financial assistance of several agribusiness corporations who, in past years, helped fund the reprinting of several issues that had gone out of print, but still were in constant demand. Their monetary help was also a psychological boost for us.

As we have said over these many years, the success of the Soybean Genetics Newsletter depends upon YOU, the soybean scientists, and we think that this volume is reason for all of you to be proud.

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Our sincere appreciation goes to those volunteers who made it all possible: April Ammons, Terry Couch, Kim Haack, Teresa Harper, Holly Heer, Telma Periera, and Paul Olson--grad students and technicians who did a lot of the detail work that always accompanies any such project.

-R. G. Palmer, editor

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SOYBEAN GENETICS COMMITTEE REPORT

FEBRUARY 1991

Minutes of the Meeting

The Soybean Genetics Committee met from 7:30 p.m. to 10 p.m., February 18, 1991, at the Memphis Marriott in Memphis TN in conjunction with the Soybean Breeders Workshop.

Committee members in attendance were T.E. Devine, T.C. Kilen, R.L. Nelson and J.R. Wilcox. It was announced that R.C. Shoemaker and R.I. Buzzell had been elected by mail ballot as the two new members to serve three year terms. At the conclusion of the meeting, J.R. Wilcox was elected Chairman for the coming year.

Also in attendance at the meeting were R.L. Bernard, J.H. Orf, Claudia Coble, Hu Yunzhu, Nanjing Agricultural University, and Wang Yantong, Chinese Academy of Agricultural Sciences. Current Committee members and the expiration dates of their terms are as follows:

J.R. Wilcox, USDA-ARS (1992) Chairman

Dept. of Agronomy
Purdue University
W. Lafayette IN 47904
(317)494-8074
FAX: (317)494-6508

S.A. Mackenzie (1993)

Dept. of Agronomy
Purdue University
W. Lafayette IN 47904
(317)494-6380
FAX: (317)494-6508

G.R. Buss (1993)

Dept. of Agronomy
Virginia Polytechnical Institute
and State University
(703)231-9788
FAX: (703)231-3431

R.L. Nelson USDA-ARS

(ex officio) (Soybean Genetics
Coll.) Dept. of Agronomy
University of Illinois
1102 S. Goodwin St.
(217) 244-4346
FAX: (217)244-7703

R.I. Buzzell (1994)

Crop Science Section
Agriculture Canada Research Station
Harrow, Ontario NOR 1G0, Canada
(519)7388-2251
FAX: (510)738-2929

R.G. Palmer USDA-ARS

(ex officio)(Curator, Cytogen-
etics Coll.; Editor, Soybean
Genetics Newsletter) Depts. of
Agronomy and Genetics, Iowa
State University, Ames IA 50011
(515)294-7378
FAX: (515)294-2299

T.C. Kilen USDA-ARS (1993)

PO Box 196
Stoneville MS 38776
(601)686-9311
FAX: (601)686-9406

R.C. Shoemaker USDA-ARS (1994)

Dept. of Agronomy
Iowa State University
Ames IA 50011
(515)292-6233
FAX: (515)292-2299

Procedure: As in the past, manuscripts concerning qualitative genetics interpretation, gene symbols and linkages should be sent to the chairman for review.

In order to facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol." Manuscripts will not be reviewed for style except as this influences the clarity of interpretation and will not be given a "peer review." Authors may submit unpolished (but comprehensible) manuscripts for review. This should reduce some of the delay involved in publishing a paper.
2. Reviewers of manuscripts will be given a deadline of two weeks to return the reviewed manuscript to the Chairman (who will then give it to the author as soon as possible). If the reviewers have not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairman.

Assignment/Approval of Gene Symbols: If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R.L. Nelson is Curator for all maturity groups. A form for this is on page 5 of this volume.

Gene symbols will be approved only in cases where the relevant material is available in one of the soybean germplasm collections for distribution to researchers. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretation does not depend upon symbols, in most cases.) The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is unnecessary.

Summaries for the Past Year: A list of the soybean gene symbols and linkages approved during the year March 1990 to February 1991 is given in Table 1. Previously approved genes that have been published recently are given in Table 2.

Committee Actions: Dr. Wilcox, chairman of the subcommittee to draft guidelines for the assessment of cytoplasmic inheritance, presented the subcommittee report to amend the guidelines. The Soybean Genetics Committee unanimously approved the subcommittee's recommendation and agreed to publish the amended guidelines with the intercalation pertinent to cytoplasmic inheritance in the forthcoming SGN.

The Committee commissioned R.L. Nelson to study and make recommendations to the Committee for suggested guidelines for the registration and addition to the genetic type collection of Maplines used for genome mapping. The Committee commissioned R.L. Nelson to study and make recommendations to the Committee for guidelines or criteria for the assessment of genetic stocks proposed for

addition to the genetic type collection. The Committee commissioned R.C. Shoemaker to study and make recommendations to the Committee for guidelines and procedures to be employed in assessing the suitability of genetic linkage data proposed for entrance into the databases for the new National Research Initiative genome mapping program.

Anyone knowing of gene symbols that do not appear in the Gene Symbol Index of the Soybean Monograph (1987, pages 192-196), or in Tables 1 and 2 in the Soybean Genetics Newsletter 16:4-5, 17:4-5, or in this issue, please advise the Soybean Genetics Committee accordingly.

All future correspondence should be sent to the new Chairman:

J. R. Wilcox, USDA-ARS
Department of Agronomy
Purdue University
West Lafayette IN 47904
(317)494-8074

T. E. Devine
Past Chairman

APPLICATION FOR ENTRY INTO THE SOYBEAN GENETIC TYPE COLLECTION

Date: _____

T number (assigned by curator): _____

Submitted by: _____

Address: _____

Return to:

R. L. Nelson, curator

USDA Soybean Germplasm Collection

Department of Agronomy

University of Illinois

1102 South Goodwin Avenue

Urbana, Illinois, 61801 U.S.A.

Strain Designation: _____

Genotype: _____

Phenotype: _____

(List the gene(s) and a description of the phenotype of the trait)

Parental Origin: _____

_____When and where found and by whom: _____

(Include year, location, institution, and name of individual making find or in charge of research).

Description: Maturity Group _____ Stem termination _____ Flower color _____

Pubescence color _____ Pubescence type and density _____ Pod color _____

Seed coat luster and color _____ Hilum color _____ Other _____

Special instructions for growing or maintenance, if any: _____
_____Literature Reference: _____

(List the reference(s) that first and best describe the discovery and inheritance of the trait. Please send relevant reprints to the curator.)

Date seedlot received at Urbana: _____ Date T number assigned: _____

Table 1. Soybean gene symbols and linkages approved March 1990 - 1991.

Date	Authors	Trait/Linkage	Gene/Linkage
Nov. 7, 1990	Fehr et al.	Palmitic acid level	<i>fap2-b</i>
June 6, 1990	Devine et al.		<i>fap?</i> (ElginEMS-421)
June 20, 1990	Hedges and Palmer	Linkage group 19	<i>Rps2 -2.7- Rj2</i>
Jan. 11, 1991	Buzzell and Anderson	Trisomic D	-
Jan. 11, 1991	Rennie et al.	Phytophthora resistance	<i>Rps1-d</i>
		Phytophthora resistance	<i>Rps?</i> (Nezumisaya)
			<i>Rps?</i> (OX939)
			<i>Rps?</i> (OX940)
Feb. 26, 1991	de Ciano and Palmer	Chlorophyll defect	<i>cyt-Y4</i>
			<i>cyt-Y5</i>
			<i>cyt-Y6</i>
			<i>cyt-Y7</i>
			<i>cyt-Y8</i>

Table 2.

Gene or Linkage	Strain	Phenotype	Reference
Linkage Group 11		<i>F-24.7-Idhl-26.9-Rjl</i>	2
<i>fapx</i>	A1937NMMU-173	Low palmitic acid	1
<i>shr</i>		Shriveled-seed	3
<i>v-2</i>	T312	Variegated leaf	4
<i>Rpg2</i>	Mcrlt	<i>Pseudomonas</i> resistance	5
<i>Rpg3</i> , <i>Rpg4</i>	Flambeau		

- 1 Fehr, W.R., G.A. Welke, E.G. Hammond, D.N. Duvick, and S.R. Cianzio. 1991. Inheritance of reduced palmitic acid content in seed oil of soybean. *Crop Sci.* 31:88-89.
- 2 Hedges, B.R., J.M. Sellner, T.E. Devine, R.G. Palmer. 1990. Assigning isocitrate dehydrogenase to linkage group 11 in soybean. *Crop Sci.* 30:940-942.
- 3 Honeycutt, R.J., J.W. Burton, R.C. Shoemaker, and R.G. Palmer. 1989. Expression and inheritance of a shriveled-seed mutant in soybean. *Crop Sci.* 29:704-707.
- 4 Honeycutt, R.J., K.E. Newhouse, R.G. Palmer. 1990. Inheritance and linkage studies of a variegated leaf mutant in soybean. *J. Hered.* 81:123-126.
- 5 Keen, N.T. and R.I. Buzzell. 1991. New disease resistance genes in soybean against *Pseudomonas syringae* pv. *glycinea*: evidence that one of them interacts with a bacterial elicitor. *Theor. Appl. Genet.* 81:133-138.

GUIDELINES ON THE EVIDENCE NECESSARY FOR THE ASSIGNMENT OF GENE SYMBOLS

Researchers are strongly encouraged to send all gene symbols and genetic interpretations to the Soybean Genetics Committee for review prior to publication to avoid duplication and/or confusion.

The following is a set of guidelines prepared by the Soybean Genetics Committee and intended to help researchers undertaking genetic analysis of soybean traits. Of necessity, these procedures will often need to be modified by the researcher to fit the specific situation, but an application of these guidelines should aid in making the correct genetic interpretation.

1. A genetic hypothesis is made on the basis of classification of segregating progeny, usually the F₂ generation and here called the hypothesis generation.
2. A second generation is classified to confirm the proposed genetic hypothesis. This second generation may be progeny of the hypothesis generation (usually F₃) or progeny of a test cross (F₁ x recessive homozygote).
3. Traits that are strongly influenced by nongenetic factors require verification of the classification scheme by evaluation of the progeny from homozygous plants of the hypothesis generation. Test cross data are not suitable for this purpose.
4. For genes controlling a phenotypic expression similar to that of previously published genes, data must be obtained to test for uniqueness and allelism. This will usually require crossing a homozygous line carrying the newly identified gene with the original sources of the previously published genes.
5. Identification of cytoplasmic factors requires reciprocal crosses between parents differing in the trait of interest. Since these factors are transmitted through the cytoplasm, the trait is expected to be associated only with the maternal parent in the F₁ and succeeding generations.
6. Conclusive evidence for cytoplasmic factors should rule out self pollinations and nongenetic factors associated with the maternal parent. Selecting parents for reciprocal crosses that differ in nuclear genetic traits (e.g., flower or pubescence color) in addition to possible cytoplasmic traits will provide evidence of cross- rather than self-pollinations by observed segregation for the nuclear genetic trait in succeeding generations.
7. Inheritance patterns in a hypothesis generation (F₂) and a confirming generation (F₃) will differentiate between cytoplasmic factors and nuclear genetic traits.
8. Follow the guidelines (Rules for Genetic symbols) published in the Soybean Genetics Newsletter to assign the symbol.

9. Submit the manuscript to the chair, Soybean Genetics Committee, for review of the genetic interpretation and approval of the gene symbol (see Soybean Genetics Newsletter for name and address).

10. If the line in which the new gene occurs is not already in the USDA Germplasm Collection, send a seed sample of the line to the curator of the Genetic Type Collection for assignment of a T-number and maintenance of the seed (see current Soybean Genetics Newsletter for name and address).

References

- Mather, K. 1951. The measurement of linkage in heredity. Methuen and Co., Ltd. Lond. John Wiley and Sons, Inc. New York.
- Hanson, W.D. 1959. Minimum family size for the planning of genetic experiments. Agron. J. 51:711-715.
- Sedcole, J.R. 1977. Number of plants necessary to recover a trait. Crop Sci. 17:667-668.

Rules for Genetic Symbols

1) Gene Symbols

- a) Gene symbols should not be assigned to traits for which no inheritance data are presented.
- b) A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below. Gene symbols may, however, be written on one line.
- c) Genes that are allelic shall be symbolized with the same base letter(s) so that each gene locus will be designated by a characteristic symbol base.
- d) Gene pairs with the same or similar effects (including duplicate, complementary or polymeric genes) should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: Y_1 , Y_2 , etc.) The numerals may be written on the same line as the base. (Example: Y_1 , Y_2 , etc.) This shall be the only use of numerals. Letter designations should not be used. The numeral 1 is automatically a part of the first reported gene symbol for each base but may be omitted only until the second symbol is assigned.
- e) The first pair of alleles reported for a gene locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: Ab , ab . Ab is allelic and dominant to ab .)
- f) If two alleles are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion and the alleles may be differentiated by adding one or two uncapitalized letters as superscripts to the base. When more than two alleles exist for a locus, the additional alleles or those symbolized subsequently to the pair first published shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: R , r^m , r .) This shall be the only use of superscripts. The letters may be written on the same line as the base if preceded by a hyphen. (For example, $Rps1-b$, $Rps1-k$, and $Ap-a$, $Ap-b$, $Ap-c$.) The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The letters may be an abbreviation of a descriptive term.

If independent mutations with the same or similar phenotype are identified at the same locus, until it is possible genetically to ascertain if they represent identical or separate alleles, the gene symbol should be followed by an identifying designation in parentheses. The identifying designation, which should NOT be in italics or underlined, can be the place where the mutation was found, the cultivar in which it was found, or any other relevant characteristic of the mutation. (Example: $ms1$ (Tonica), or $ms1$ (Ames 2).) This will ensure that possible subtle differences between the mutations, such as differences in DNA sequence, or unique pleiotropic side effects, are not overlooked by workers using those genes.

- g) Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.
- h) The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clear-cut. The decision for intermediate cases is at the discretion of the author, but should be in accordance with previous practices for the particular type of trait.

The following sections concern supplementary symbols that may be used whenever desired as aids to presentation of genetic formulas.

- i) An underscore may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A₋ represents both AA and Aa.)
- j) A question mark may be used in place of a symbol when the locus or allele is unknown or doubtful. The name of the line in which the gene was identified should be included in the symbol, in parentheses. A hyphen preceding the question mark indicates an unknown allele at a known locus, the absence of a hyphen indicates an unknown locus. (Example: Rps? (Harosoy) an allele in Harosoy at an unknown locus or Ap-? (Tl60) an unknown allele in Tl60 at the Ap locus.
- k) Plus symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

II) Isoenzyme Symbols and Protein Gene Symbols

The following set of guidelines is to be used when assigning gene symbols to isoenzyme variants. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybeans.

- a) A gene symbol (generally three letters) that indicates, as clearly as possible, the name of the enzyme should be used. The example, Adh (alcohol dehydrogenase); Idh (isocitrate dehydrogenase). The appropriate Enzyme Commission name and number should be used in the original article, when appropriate, to designate the specific enzyme activity being investigated.
- b) The electrophoretic conditions used to characterize a locus or allele should be specified clearly and in sufficient detail to be repeated by others interested in using the locus in genetic studies. The electrophoretic mobility, or other properties of an allele, should be clearly described by the authors.
- c) Publications should include a photograph and/or an interpretive zymogram that allows readers to visualize the variability described by the authors, as well as to ensure that subsequent work corresponds to the original study.

III) Linkage and Chromosome Symbols

- a) Linkage groups and the corresponding chromosomes shall be designated with arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.
- b) Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc. The symbol Def shall denote deficiencies, Inv inversions, and Tri primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second as Def 1b, etc. The first published inversion in chromosome 1 shall be designated with the arabic numeral that corresponds to its respective linkage group number.
- c) Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2, the second case, etc. The first published deficiency shall be symbolized as Def A, the second as Def B, etc. The first published inversion shall be symbolized as Inv A, and the second as Inv B, etc. The first published trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

(V) Cytoplasmic Factor Symbols

- a) Cytoplasmic factors shall be designated with one or more letters prefixed by cyt-. (Example: cyt-G indicates the cytoplasmic factor for maternal green cotyledons, cyt-Y indicates that for maternal yellow cotyledons.)
- b) Designations for specific cytoplasmic factors, following cyt-, shall follow the same format as for gene symbols. Base letters chosen to indicate apparent relationships among traits will have common initial letters for all loci in a related group of traits. Initial letters will be consistent with initial letters designating nuclear gene traits. (Example: cyt-G green seed embryo, cyt-Y2 yellow leaves, becoming yellowish green.)

V) Priority and Validity of Symbols

- a) A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the

existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.

- b) In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

V) Rule Changes

- a) These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

USDA Soybean Genetic Collection: Type Collection

The Soybean Genetic Type Collection is generally comprised of strains expressing known or putative qualitative traits that are not included in any other portion of the USDA Soybean Germplasm Collection. Lines containing genes with symbols newly assigned by the Soybean Genetics Committee are deposited in the Type Collection if the gene is not already in the USDA Soybean Germplasm Collection. There are currently 141 accessions in the Type Collection. These strains are assigned T-numbers in chronological order as submitted. Where previous strain designations have been used, they are given in parentheses under the T-number. Most of the T-strains are mutants. Those that are segregants from crosses or presumed outcrosses are marked with a "*" after the genotype. For T-strains with an H suffix (e.g., T211H) the allele is carried as the heterozygote because the homozygote is lethal, sterile, or very weak. For T-strains with a M suffix (e.g., T225M) the trait is maintained by selecting the mutable genotype. Cytoplasmically inherited traits are prefixed by *cyt*-. Genes for secondary traits of interest are listed in parentheses. Numerical superscripts are used to indicate backcrosses; e.g., Lincoln² x Richland is equal to Lincoln x (Lincoln x Richland). A packet of 50 seeds of any of these strains are available for research purposes from the USDA Soybean Germplasm Collection, University of Illinois, Department of Agronomy, W-321 Turner Hall, 1102 South Goodwin Avenue, Urbana, Illinois, 61801, USA.

Strain	Genotype	Phenotype	Parental origin	When and where found
T16	--	Brown hilum on black seed	Ebony	Before 1930 at Urbana, IL
T31	<i>p2</i>	Puberulent	Soysota x Ogemaw	1926 by F.W. Wentz at Ames, IA
T41	<i>1n (d1 d2)*</i>	Narrow Leaflet	Unknown	Before 1930 at Urbana, IL
T43 (Progeny 435B)	<i>P1 (cyt-G1)</i>	Glabrous	Medium Green x "glabrous"	Before 1927 at Urbana, IL
T48	--*	Spread hilum	Manchu x Ebony	Before 1930 at Urbana, IL
T54	<i>dt1*</i>	Determinate stem	Manchu	Before 1927 at Urbana, IL
T93	<i>v1 (D1 d2 or d1 D2)</i>	Variegated leaves	Hybrid population	Before 1931 at Urbana, IL
T93A	<i>v1 (d1 d2)</i>	Variegated leaves	T93	At Urbana, IL
T102	<i>y4 1e</i>	Greenish yellow leaves, weak plant; seed lectin absent	Wilson-Five	Before 1932 at Urbana, IL
T104	<i>d1 d2 G cyt-G1</i>	Green seed embryo, green seed coat	T42 (green cotyledon from H. Terao) x "Chromium green"	Before 1932 at Urbana, IL
T116H	<i>y5</i>	Greenish yellow leaves, very weak plant	Radium-treated PI 65.388	Before 1934 at Urbana, IL

Strain	Genotype	Phenotype	Parental origin	When and where found
T117 (L34-602)	<i>Dt2 lw1 Lw2*</i>	Semi-determinate stem, non-wavy leaf	AK114 x PI 65.394	Before 1934 at Urbana, IL
T122	<i>lo (d1 d2)</i>	Oval leaflet, few-seeded pods	Unknown	Before 1934 at Urbana, IL
T134	<i>y5</i>	Greenish yellow leaves	Illini x Peking	1937 at Urbana, IL
T135	<i>y9</i>	Bright greenish yellow leaves	Illini	1938 at Urbana, IL
T136	<i>y6 (ln dt1)</i>	Pale green leaves	PI 88.351 x Rokusun	1937 at Urbana, IL
T138 (L35-1156)	<i>y7 y8</i>	Yellow growth in cool weather	Unknown	Before 1935 at Urbana, IL
T139	<i>g y3*</i>	Yellow seed coat, leaves turn yellow prematurely	Illini	About 1936 by Brunson in Kansas
T143	<i>Lf1 g y3 y7 y8*</i>	5-foliate, leaves turn yellow prematurely, and in cool weather	T138 x T137 (T137 is <i>y3</i> from a cross in PI 81.029)	By 1935 at Urbana, IL
T144	<i>d1 d2 v1 y7 y8*</i>	Green seed embryo, vari- egated leaves, yellow growth in cool weather	LX431 (T93A x T138)	At Urbana, IL
T145 (9-776)	<i>Pl*</i>	Glabrous	Unknown	At Urbana, IL
T146	<i>r-m*</i>	Brown seed with black stripes	LX286 (PI 82.235 x PI 91.073)	At Urbana, IL
T152	<i>i</i>	Self dark seed coat	Lincoln	By 1938 at Urbana, IL
T153	<i>k1</i>	Dark saddle on seed coat	Lincoln	By 1938 at Urbana, IL
T157	<i>i</i>	Self dark seed coat	Richland	By 1938 at Urbana, IL
T160	--	Pale green leaves	Hahto (Michigan)	By 1938 at Urbana, IL
T161	<i>y10</i>	Greenish yellow seedling	L36-5 from Mandarin x Mansoy	1940 at Urbana, IL

Strain	Genotype	Phenotype	Parental origin	When and where found
T162	<i>yl7</i>	Light yellowish-green leaves	Mandarin	1940 at Urbana, IL
T164	--	Slightly variegated leaves	Morse	1941 at Urbana, IL
T171	--*	Long peduncle	Unknown	At Urbana, IL
T173	<i>f (ln)*</i>	Fasciated stem	Keitomame (<i>f</i>) x PI 88.351 (<i>ln</i>)	At Urbana, IL
T175	<i>El t*</i>	Late maturity, gray pubescence	Unknown	At Urbana, IL
T176	<i>lw1 lw2 (Dt2)*</i>	Wavy leaf	Unknown	At Urbana, IL
T180 (L46-1741-2)	<i>Rj1*</i>	Nodulating	Same F ₃ plant as T181	At Urbana, IL
T181 (L46-1743-2)	<i>rj1*</i>	Nonnodulating	Lincoln ² x Richland	At Urbana, IL
T201	<i>rj1*</i>	Nonnodulating	LX1277 (L46-1743 x L46-1741)	At Ames, IA
T202	<i>Rj1*</i>	Nodulating	Sib of T201	At Ames, IA
T204 (L48-101)	<i>ln lo*</i>	Narrow leaflet, 4-seeded pods; oval leaflet, few-seeded pods	T136 x T122	At Urbana, IL
T205 (L48-163)	<i>lw1 lw2*</i>	Wavy leaf	Dunfield x Manchuria 13177	At Urbana, IL
T208 (Ind. Acc. 2300-2)	<i>Se*</i>	Pedunculate inflorescence, small seeds	PI 196.176* ('Yu Tae' from Korea)	
T209 (L50-155)	--*	Dwarf?	Lincoln x "wild dwarf"	At Urbana, IL
T210 (L49-738)	<i>df2</i>	Dwarf	Colchicine-treated Lincoln	At Urbana, IL
T211H (CX3941-844-2-5)	<i>pm</i>	Dwarf, crinkled leaves, sterile	Kingwa x T161	At Lafayette, IN

* Not in USDA Southern Soybean Germplasm Collection, considered a duplicate of PI 196.177.

Strain	Genotype	Phenotype	Parental origin	When and where found
T216 (L46-266)	--*	Reddish black seeds	PI 86.038 x PI 88.351	1946 at Urbana, IL
T218M	Y18-m	Chlorophyll chimera, (resembles T225M)	Illini	1952 at Urbana, IL
T219H (A691-1)	y11	Lethal yellow, (hetero- zygote has greenish yellow leaves)	Richland x Linman 533	1941 at Ames, IA
T220 (L46-431)	--	Greenish yellow leaves	Lincoln	At Urbana, IL
T221 (L46-426)	--	Yellowish green leaves	Peking	At Urbana, IL
T223 (L46-429)	--	Yellowish green leaves	Richland	At Urbana, IL
T224 (L46-428)	--	Greenish yellow leaves	Richland	At Urbana, IL
T225M	Y18-m	Unstable gene resulting in chlorophyll chimera	Lincoln	Before 1955 in Iowa
T225H	y18	Near-lethal yellow leaves	T225M	
T226	--	Greenish yellow leaves	Lincoln	1943 at Ames, IA
T227	--	Greenish yellow leaves, becoming green	Illini	1943 at Kanawha, IA
T229	y14	Light green leaves	F, Richland x Linman 533	1943 at Ames, IA
T230 (A43K-643-1)	y13	Whitish green seedling, greenish yellow leaves	Mandell x Mandarin (Ottawa)	1944 at Kanawha, IA
T231 (A49-8414)	--	Greenish yellow leaves, weak plant	AX3015-55 (Richland x Linman 533)	1943 at Ames, IA
T232	--	Yellowish green leaves	Hawkeye	1950 at Ames, IA
T233	y12	Whitish primary leaves, yellowish green leaves	Hawkeye	1950 in field N2100 at Ames, IA
T234	y15	Pale yellowish green leaves	L46-2132 (Clark progenitor)	1952 at Ames, IA

Strain	Genotype	Phenotype	Parental origin	When and where found
T235 (L58-274)	<i>wm</i>	Magenta flower	Harosoy	1957 at Urbana, IL
T236 (L46-232)	(<i>Lf1 ln y6</i>)*	Red-buff seed	T143 x " <i>y6 ln pc dt1 w1</i> "	1946 at Urbana, IL
T238 (S57-3416)	<i>k3</i>	Dark saddle on seed coat	X-rayed Clark	1956 at Columbia, MO
T239 (L63-365)	<i>k2</i>	Tan saddle on seed coat	Harosoy	1961 at Urbana, IL
T241H	<i>st2</i>	Asynaptic sterile	S54-1714 (from same cross as Wayne)	About 1956 at Columbia, MO
T242H	<i>st3</i>	Asynaptic sterile	AX54-118-2-8 (Blackhawk x Harosoy)	At Lafayette, IN
T243	<i>df2</i>	Dwarf	Colchicine-treated Lincoln	At Ames, IA
T244 (Adams 77-2)	<i>df3</i>	Dwarf	Neutron-irradiated Adams	At Ames, IA
T249H (L67-4408A)	--(<i>P1</i>)	Whitish yellow seedling, lethal	F ₃ (Clark ⁶ x PI 84.987) x (Clark ⁶ x T145)	1964 at Urbana, IL
T250H (L67-4439)	--	Lethal seedling	F ₂ Harosoy ⁵ x (Clark ⁶ x Chief)	1964 at Urbana, IL
T251H (L67-4440A)	<i>mn</i>	Miniature plant	F ₂ Harosoy ⁵ x T139	1961 at Urbana, IL
T252 (L64-2612)	--	Pale green leaves	F ₃ Harosoy ⁶ x T139	1963 at Urbana, IL
T253 (L67-4415A)	<i>y20 (k2)</i>	Yellowish green leaves, weak plant	T239	1963 at Urbana, IL
T254 (L67-4412A)	--	Greenish yellow leaves	F ₂ Clark ⁶ x T176	1964 at Urbana, IL
T255	<i>lf2</i>	7-foliolate	Hawkeye	1966 at Ames, IA
T256	<i>df4</i>	Dwarf	Hark	1966 at Ames, IA

Strain	Genotype	Phenotype	Parental origin	When and where found
T257H	<i>y16</i>	Near-lethal white	C1128 ^a x Mukden (C1128 is from Wabash x Hawkeye)	At Lafayette, IN
T258H (A72-1103-6)	<i>st4</i>	Desynaptic sterile	Hark	1968 at Ames, IA
T259H (L71L-06-4)	<i>ms2</i>	Male sterile	F ₃ of SL11 (Wayne-r 1971 at <i>Rpm Rps1</i>) x L66L-177 Eldorado, (Wayne x (Hawkeye x IL Lee))	IL
T260H (N69-2774)	<i>ms1</i> (North Carolina)	Male sterile	Unknown	1966 in a farmer's field in N. Carolina
T261 (S56-26)	<i>k2</i>	Tan saddle on seed coat	Mandarin (Ottawa)	Before 1956 at Columbia, MO
T262	--	"Double pod"	SRF 200 (Hark-1n)	About 1971 at Soybean Research Foundation, Mason City, IL
T263 (A76-2)	<i>df5</i>	Dwarf	Harosoy 63 x PI 257.435	1968 at Iowa State Univer- sity nursery, Hawaii
T264 (L58-2749)	<i>Pd2</i>	Dense pubescence	Neutron-irradiated Blackhawk in the M ₂ generation	1956 at Urbana, IL
T265H (L75-0324)	<i>y19</i>	Delayed albino	F ₂ Williams ⁶ x T259	1974-75 greenhouse at Urbana, IL
T266H	<i>ms1</i> (Urbana)	Male sterile, (higher female fertility than T260, T267, and T268)	F ₃ row of L67-533 (Clark ⁶ x Higan) x SRF 300	1971 at Urbana, IL
T267H (L56-292)	<i>ms1</i> (Tonica)	Male sterile	Semisterile plant found in Harosoy	1955 by F.M. Burgess at Tonica, IL
T268H (A73g-21)	<i>ms1</i> (Ames 1)	Male sterile	Semisterile plant found in T258H	1970 at Ames, IA

Strain	Genotype	Phenotype	Parental origin	When and where found
T269H (L70-8654)	<i>fs1 fs2</i>	Structural sterile (T269H is from <i>Fs1 fs1 fs2 fs2</i> plants)	Flower structure mutant segregating in a plant-progeny row from PI 339.868	1970 at Urbana, IL
T270H (A78-286)	<i>y22</i>	Greenish yellow leaves, very weak plant	Segregating in an F_2 -plant-progeny row from an outcross in T271H	1977 at Ames, IA
T271H	<i>msp</i>	Partial male sterile	40-parent bulk population (AP6 (S1)C1)	1975 at Ames, IA
T272H (A71-44-13)	<i>st5</i>	Desynaptic sterile	Uniform Test entry W66-4108 from Merit x W49-1982-32 (W49-1982-32 is from Hawkeye x Manchú 3)	1970 at Ames, IA
T273H (A72-1711)	<i>ms3</i>	Male sterile	Semisterile plant in an F_3 -plant-progeny from Calland x Cutler	1971 at Washington, IA
T274H (A74-4646)	<i>ms4</i>	Male sterile	Semisterile plant in Rampage	1973 at Ames, IA
T275 (A77-K150)	<i>cyt-Y2</i>	Yellowish leaves, becoming greenish yellow	Chimeric F_2 plant A75-1165-117 from T268H x (PI 101.404B x Clark ⁶)	1975 at Ames, IA
T276	<i>nrl</i>	Constitutive nitrate reductase absent	M_2 generation of Williams treated with EMS, nitroso-guanidine, & X-rays	1979 at Urbana, IL
T277H	<i>ms5</i>	Male sterile	Semisterile plant in the M_2 generation of neutron-irradiated Essex	1976 at Blacksburg, VA
T278M	<i>cyt-Y3</i>	Yellow leaves, very weak plant (mutable plants are chlorophyll chimeras)	Chimeric plant of unknown source	1972 at Ames, IA

Strain	Genotype	Phenotype	Parental origin	When and where found
T279 (D76-1609)	<i>lps*</i>	Short petiole	F ₃ (Forrest ¹ x Sodendaizu (PI 229.358)) x D71-6234 (D71-6234 is a selection from a high protein Lee type x PI 95.960)	1976 at Stoneville, MS
T280 (C1640)	<i>fan</i>	Low linolenic acid	Century treated with mutagenic agent ethyl methanesulfonate. C1640 is the progeny of a 1981 M ₂ plant	1981 at West Lafayette, IN
T281 (L58-617)	--	Dwarf plant, rugose leaf	Offtype in PI 232.992	1955 at Urbana, IL
T282 (L81-5482)	--	Curled leaf	Abnormal mutant or segregant in F ₃ of Williams x PI 82.278	1980 at Urbana, IL
T283 (A77-86)	--	Chlorophyll deficient	F ₇ plants of PI 101.404B x Clark ⁶	1977 at Ames IA
T284H	<i>ms3</i> (Flanagan)	Male sterile	Outcrossed male-sterile plant in Wabash	1973 by H.K. Chaudhari and W.H. Davis
T285 (IL3-1)	<i>fr5</i>	Nonfluorescent seedling	Williams treated with gamma rays	1981-1984 at Ames, IA
T286 (MS2060)	<i>df6</i>	Dwarf	C1421 (Adelphia ⁸ x Mukden) treated with EMS	Early 1980's at W. Lafayette, IN
T287H (S85-62-11)	<i>ms1</i> (Ames 2)	Male sterile	Segregating in S ₄₅ progeny from AP6(S1)C1	1984 by R. Secrist at Ames, IA
T288 (Williams 80-7)	<i>y23</i>	Leaves becoming yellow-white and necrotic, viable plant	Williams	1980 by A.K. Williams at Williams, IN
T289 (Hardee 2)	<i>Got-c</i>	Glutamate oxaloacetic transaminase variant	Hardee	1983 by Y.T. Kiang at Durham, NH
T290H	<i>ms1</i> (Danbury)	Male sterile	Beeson outcross	Prior to 1988 by M.C. Albertsen at Danbury, IA

Strain	Genotype	Phenotype	Parental origin	When and where found
T291H	<i>ms3</i> (Plainview)	Male sterile	F ₂ (Viking x Classic II) x (Mitchell x Columbus) 'Viking' is a private line from Merit x Amsoy	Prior to 1988 by W.H. Davis at Plainview, TX
T292H	<i>ms4</i> (Fisher)	Male sterile	Corsoy	Prior to 1988 by W.H. Davis at Fisher, AR
T293 (Altona-spl)	<i>spl</i>	β -amylase null	Altona	Prior to 1978 by M.B. Gorman and Y.T. Kiang at Durham, NH
T294 1982 by (G81-6299)	<i>g3</i>	green seed coat	F ₆ Duocrop x G76-57. G76-57 is from Bragg x Kent.	line from In H.R. Boerma at Athens, GA
T295H	<i>ms6</i>	Male sterile, female fertile	Line 245014 from A74-204034 x C1520	In 1978 by W.R. Fehr at Ames, IA
T296	--	5 to 7 foliolate	Williams x BB 13 No.9, which was a mutant in 'SJ2'	BB 13 No. 9 found in 1976 by A. Waranyuwat, Northeast Agric. Center, Khonaen, Thailand
T297H (L88-3785)	--	Semi-sterile	F ₇ row from Clark x PI 317.334B	In 1984 by R.L. Bernard at Urbana, IL
T298H (L88-3809)	--	Near sterile	F ₃ row from L64-2887(Clark-i) ⁶ x Sooty	In 1982 by R.L. Bernard at Urbana, IL
T299H (L88-3834)	-- (eu)	Sterile	F ₃ row from Williams ⁶ x PI 229.324	In 1985 by R.L. Bernard at Urbana, IL

Strain	Genotype	Phenotype	Parental origin	When and where found
T300H (L88-3854)	--	Sterile	F ₁ row from L78-375 (Williams-Rsv) x PI 86.740	In 1985 by R.L. Bernard at Eldorado, IL
T301H (L88-3886)	--	Semi-sterile	F ₁ row from Beeson x Prize	In 1981 by R.L. Bernard at Urbana, IL
T302H (L88-3962)	--	Near sterile	Beeson	In 1973 by R.L. Bernard at Urbana, IL
T303H (L88-3966)	--	Near sterile	L73-5446 from L67-1250 (Harosoy-Dt2) x L62-1251 (Clark-Dt2)	In 1975 by R.L. Bernard at Urbana, IL
T304H (L88-3981)	--	Near sterile	L73-5741 from Corosy x Amsoy 71	In 1975 by R.L. Bernard at Urbana, IL
T305H (L88-4064)	--	Semi-sterile	L75-12103 from Wells x Williams	In 1975 by R.L. Bernard at Urbana, IL
T306H (L88-4106)	--	Sterile	PI 506.669 (<i>'Fujihime'</i>)	In 1987 by G.A. Juvik at Urbana, IL
T307 (A5)	<i>fan</i> (A5)	Low linolenic acid	EMS mutant of FA 9252 (PI 80.476 x PI 85.671)	In 1980 by W.R. Fehr and E.G. Hammond at Ames, IA
T308 (C1726)	<i>fap1</i>	Low palmitic acid	Ems mutant of Century	In 1982 by J.R. Wilcox, W. Lafayette, IN
T309 (C1727)	<i>fap2</i>	High palmitic acid	Ems mutant of Century	In 1982 by J.R. Wilcox, W. Lafayette, IN
T310 (L81-4148)	--	Weak stem, buff seed coat, and wavy leaflet margin	Harosoy x L67-3391 (L67-3391 was a mutant found in Harosoy)	In 1965 by R.L. Bernard at Urbana, IL

Strain	Genotype	Phenotype	Parental origin	When and where found
T311	<i>shr</i>	Shriveled seed	F ₆ generation of AP2 x P2180	In 1982 by C. Jennings & R. Freestone, Cedar Falls, IA
T312	<i>v2</i>	Variegated leaves	Clark	In 1978 by K. Newhouse, Ames, IA
T313	<i>lnr</i>	Narrow, rugose leaflet	EMS treated C1421	In 1975 by J. Wilcox, W. Lafayette, IN
T314		Yellow leaves	Variant in progeny of a chimeric plant from F ₃ Clark x T251	In 1981 by R. Palmer, Ames, IA
T315	<i>cyt-4</i>	Vigorous plant with yellow leaves	Variant in Williams	In 1981 by A. Williams, Williams, IN
T316	<i>cyt-5</i>	Yellow leaves	Variant in F ₆ [(Corsoy x Rampage) x Franklin]	In 1982 by R. Freestone, Waterloo, IA
T317	<i>cyt-6</i>	Yellow plant missing 2 malate dehydrogenase bands	Somaclonal mutant in Jilin 3 (PI427.099)	In 1988 by L. Amberger, Ames, IA
T318	--	Aconitase 2 variant	Somaclonal mutant in BSR 101	In 1988 by L. Amberger, Ames, IA
T319	<i>cyt-7</i>	Weak plant with yellow leaves	Chimeric plant in AX2950 (Hack x 407.298)	In 1985 by G. Graef, Ames, IA
T320	<i>cyt-8</i>	Greenish-yellow leaves	F9 chimeric plant from Williams x Essex cross	In 1986 by E. Roberts, St. Joseph, IL
T321	<i>w4-dp</i>	Pale purple throat flower	F11 plant from w4-mutable line	In 1986 by R. Groose, Ames, IA

The following table gives the maturity group and some descriptive data for the strains in the Type Collection.

Descriptive Code Sequence: 1 2345 678 (underlined letters used in code)

1. Stem Termination: Indeterminate, Semi-determinate, Determinate
2. Flower Color: Purple, Light purple, White, Magenta, Purple throat
3. Pubescence Color: Tawny, Light tawny, Gray
4. Pubescence Form: Appressed, Curly, Erect, Irrregular, Semi-appressed, Puberulent
5. Pubescence Density: Dense, Glabrous, Normal, Semi-dense, Semi-spars
6. Pod Color: Black, Brown, Dark brown, Tan
7. Seed Coat Luster: Pull, Shiny, Intermediate, Bloom
8. Seed Coat Color: Yellow, Green, Gray, Black, Brown, Tan, Buff
Imperfect black,
May have prefix Light, Dark, or Reddish.
9. Hilum Color: Same abbreviations as seed coat color

Strain	Maturity Group	Descriptive Code	Strain	Maturity Group	Descriptive Code
T16	IV	N WTESspTn SB1Br	T173	IV	D WGENTn SYY
T31	IV	D P_PtNBr SRbrRbr	T175	IV	D WGESspTn DBfBf
T41	IV	D WTENBr SGnG	T176	II	S PGENBr SYIb
T43	V	D W_GBr DGnBr	T180	IV	N WTENBr DYB1
T48	IV	S WTENBr SYB1	T181	IV	N WTENBr DYB1
T54	III	D PTENBr SYBr	T201	IV	N WTENBr SYG
T93	I	N PTENBr SGnB1	T202	IV	N WTENBr SYG
T93A	II	N WTENB1 DGnG	T204	IV	D WGENTn DBfBf
T102	III	N PGENTn SB1B1	T205	IV	N PGENBr SYIb
T104	IV	D PTESspBr SGnB1	T208	IV	D PGENTn DYBf
T116H	II	N PTASspBr SBrBr	T209	III	D PTENBr SGG
T117	IV	S PGENBr DYIb	T210	IV	N WTSaSpBr SYB1
T122	IV	D WGENTn DBfBf	T211H	IV	N WLtENBr SGnB1
T134	III	N WGENBr SYBf	T216	IV	N WGESspBr SRb1Rb1
T135	III	N WGENBr SYBf	T218M	III	N WGENBr SYBf
T136	IV	D WGESspBr DYBf	T219H	III	N PTENBr DYG
T138	IV	N PTENBr SYB1	T220	III	N WTENBr SYB1
T139	III	N WGENBr SYBf	T221	IV	N WTENTn SB1B1
T143	III	N WTENBr SB1B1	T223	II	N PGENBr DYG
T144	IV	N PTENBr SGnB1	T224	II	S PGENBr DYG
T145	III	D W_GTn SBrBr	T225H	III	N WTENBr SYB1
T146	IV	N PTENBr DBrBr	T225M	III	N WTENBr SYB1
T152	III	N WTENBr SB1B1	T226	IV	N WGENBr SYBf
T153	III	N WTENBr SYB1	T227	III	N WGENBr SYBf
T157	III	S PGENBr DIbIb	T229	IV	N PTENBr DYB1
T160	IV	D PTENBr SGnBr	T230	III	N PGENBr SYY
T161	IV	N WTENBr SYB1	T231	IV	N PTENBr DYB1
T162	I	N PGENBr SYY	T232	III	N PGENBr DYIb
T164	IV	D WGENBr SGnGn	T233	III	N PGENBr DYIb
T171	IV	D WTENBr SYG	T234	IV	N PTENBr SYB1

Strain	Maturity Group	Descriptive Code	Strain	Maturity Group	Descriptive Code
T235	II	N MGENBr DYY	T281	I	N PGENTn DYg
T236	II	N W_CNTn IRbfrbf	T282	IV	N WTESspTn IGgnBl
T238	IV	N PTENBr DYBl	T283	IV	N PTENBr DYBl
T239	II	N PGENBr DYTn	T284H	IV	D PGENTn SYIb
T241H	IV	N WTENBr SYBl	T285	III	N WTENTn IYBl
T242H	II	N PGENBr DYg	T286	III	N WGENTn SYBf
T243	IV	N WTSaSpBr SYBl	T287H	II	N PTENBr DYBl
T244	III	N WGENTn SYBf	T288	IV	N WTENTn SYBl
T249H	IV	N P_GBr DYBl	T289	VIII	WG IYBf
T250H	III	N PGENBr DYY	T290H	III	N PTENBr DYBl
T251H	II	N PGENBr SYY	T291H	III	N PGENBr IYIb
T252	II	N PGENBr DYY	T292H	II	S PGENBr DYg
T253	III	N PGENBr DYTn	T293	00	N PTENBr SYBl
T254	IV	N PTENBr DYBl	T294	VII	N WTENBr SGBl
T255	II	N PGENBr DYIb	T295H	II	N WTENBr IYBl
T256	IV	N PGENBr DYY	T296	>IV	D WTENTn IYBl
T257H	III	N WGENTn SYBf	T297H	II	N PTENBr DYBr
T258H	II	N PGENBr DYY	T298H	III	N PTENBr BB1Bl
T259H	III	N WTENTn DYY	T299H	III	N WTENTn IYBl
T260H	VII	D WGENBr SYBf	T300H	III	N WTENTn SYBr
T261	I	N PGENBr SYTn	T301H	II	N PGENBr DYY
T262	IV	N PGENBr DYY	T302H	II	N PGENBr DYIb
T263	I	N PGENBr DYY	T303H	II	S PGENBr DYg
T264	I	N WGEDnBr SYBf	T304H	I	N PGENTn SYY
T265H	III	N WTENTn SYBl	T305H	II	N WTENTn DYBl
T266H	IV	N PTENBr IYBl	T306H	II	D PTESspBr SYLbr
T267H	II	N PGENBr DYY	T307	0	N PTENBr SYBl
T268H	II	N PGENBr DYY	T308	II	N PTENBr SYBl
T269H	III	D WGANTn DYBf	T309	II	N PTENBr SYBl
T270H	IV	N PTENBr DYBl	T310	II	N PGENBr DBfBf
T271H	II	N PTENBr DYBl	T311	I	N WGENBr DYBf
T272H	I	N WTESspBr DYBl	T312	III	N PTENBr DYBl
T273H	IV	N PTENBr DYBl	T313	III	N WGENTn SYBf
T274H	I	N PTENBr SYBl	T314	III	N PGEN_ IYg
T275	IV	N PTENBr DYBl	T315	III	N WTEN_ IYBl
T276	III	N WTENTn SYBl	T316	III	N PGEN_ IYg
T277H	V	D PGENTn IYIb	T317	I	N PGEN_ SYLbF
T278M	III	N PGENBr DYY	T318	I	N PGEN_ DYIb
T279	VII	D WTSaNBBr IYBl	T319	II	N PTEN_ IYBl
T280	II	N PTENBr DYBl	T320	IV	N WTEN_ IYBl
			T321	II	N LpthGENBr DYBf

Randall L. Nelson, USDA-ARS
 Richard L. Bernard, University of Illinois

USDA Soybean Genetic Collection: Isoline Collection

The U.S. Department of Agriculture Soybean Germplasm Collection at the University of Illinois, Urbana, maintains sets of near-isogenic lines of soybeans as described in the following list. These lines were developed primarily by backcrossing to the adapted commercial varieties Clark (maturity group IV), Harosoy (group II), and Williams (group III) as recurrent parents. Many of the isolines were listed in 1975 in SCN 2:59-74 and are included here to provide the researcher with a complete list in one document. The lines released since 1975 are indicated with a hyphen preceding the line designation. They include many new combinations and additional genes, and a series of isolines involving Williams as the recurrent parent.

These isolines originated as plant selections from the indicated backcrosses (usually BC5), combinations between such lines, or mutants. They are homozygous for the indicated genes (except for lethal and sterile genes) and have been selected for similarity to the recurrent parent for other traits. They involve over 60 different nuclear genes and cytoplasmic factors, which may be useful in genetic and other research. Some have a demonstrated or potential use in commercial variety development. The attached list only briefly describes the gene effects, and reference should be made to pertinent technical literature for more details.

A list of references to gene symbols:

Palmer, R.G., and T.C. Kilen. 1987. Qualitative Genetics and Cytogenetics, Chapter 4 in J.R. Wilcox, ed. Soybeans: Improvement, Production, and Uses (2nd ed.). pp. 139-209. American Society of Agronomy.

Information on origins of domestic and foreign cultivars used as parents is in the following:

Bernard, R.L., G.A. Juvik, and R.L. Nelson. 1987 and 1989. USDA Soybean Germplasm Collection Inventory, vol. 1 and 2. 86 and 203 pp. INTSOY Series No. 30 and 31, USDA-ARS and University of Illinois, Urbana-Champaign. (Old domestic varieties, FC strains, and PI strains to PI 150,000 are in vol. 1; PI 150,000 to PI 500,000 are in vol. 2.) A description of the T strains is given elsewhere in this publication.

Bernard, R.L., G.A. Juvik, E.E. Hartwig, and C.J. Edwards. 1988. Origins and Pedigrees of Public Soybean Varieties in the United States and Canada. 68 pp. USDA-ARS Technical Bulletin 1746. (domestic varieties released by 1945 and public varieties 1946 to 1986)

In the following list of isolines the LINE column indicates the designation of the specific homozygous line available, usually an F2 or F3 plant progeny. The strain designation in parentheses is for a bulk of similar lines with the same genotype, which may have been in Uniform Tests, released, or in the pedigrees of other lines. Thus L61-4222(L7) means that L61-4222 is a subline of L7, L60-246(Clark 63) means that L60-246 is a subline of Clark 63, etc. In some cases a sister isolate (from the same cross) has been substituted for a line previously released or used in crosses, because it appeared more nearly isogenic to the recurrent parent. The previous line's designation is given in parentheses preceded by "for". For example, L79-1800(for L74-387) indicates that L79-1800 has been substituted for L74-387, which is no longer maintained. In some cases where two similar isolines have been widely used or the final choice of a single isolate has not yet been made, both are listed and maintained, as in L62-1932, L63-3117 for Clark-e2.

Disease resistant isolines were sometimes used in place of the commercial varieties as recurrent parent. The major recurrent parents, their abbreviations and descriptions, and sublines used in backcrossing are as follows:

Clark (C) Usually the typical subline L58-231 (formerly designated Clark-L1).
 L6 A phytophthora and pustule resistant Clark isoline with genes *Rps1* and *rxp* and apparently isogenic to Clark 63; usually subline L61-5448.
 L12 L6 plus yellow hilum (genes *I* and *r*); usually subline L64-2244.

Harosoy (H) Usually the typical subline L58-266 (formerly designated Harosoy-L2).
 L2 Phytophthora and pustule resistant Harosoy isoline with genes *Rps1* and *rxp*; usually subline L61-5047.

Williams (Wm) Bulk breeder seeds were used in crossing.
 Williams 79 (Wm 79) Williams-*Rps1*-c. Usually the typical subline L75-3735.
 Williams 82 (Wm 82) Williams-*Rps1*-k. Usually the typical subline L77-1779 or 1794.
 Will Williams-*Dt2*. Usually the typical subline L74-189.

Many isolines have genes *Rps1* and *rxp* from L2, L6, L10, or L12 and this is indicated by an asterisk(*) in the GENE column. ** indicates that the line is probably *Rps1 rxp* but the testing is not completed. In the case of Wayne backcrosses * indicates the presence of *Rpm Rps1* from SL12 (original Wayne and all of its isolines are *rxp*).

In the PARENTAGE column the number in parentheses is the number of crosses to the recurrent parent. When isolines are being combined the pertinent gene symbols are often inserted in the parentage to indicate their source. Thus the parentage of L67-3207 (Clark-*dt1 s-t*), which is (C(6) x Chief) x L63-3297, is written (C(6) x Chief)*s-t* x L63-3297,*dt1*.

The numbers of isolines (excluding duplicates) by recurrent parent are:

Clark	270	Chippewa	11	Wells	1
Harosoy	124	Corsoy	5	PI 65.549	1
Amsoy 71	2	Elf	1	Williams	62
Beeson	1	Wayne	14	Total	490

A seed packet (50 seeds, or more if available) of each line will be made available for research purposes upon request to USDA Soybean Germplasm Collection, W-321 Turner Hall, 1102 South Goodwin Ave., Urbana, Illinois 61801. It is requested that appropriate recognition be made if this germplasm contributes to the development of a new cultivar or when research is published using these isolines.

R. L. Bernard, University of Illinois
 R. L. Nelson, USDA-ARS
 C. R. Cremeens, USDA-ARS

GENE(S)*LINEPARENTAGE

CLARK ISOLINES

L58-231(Clark)

Lincoln(2) x Richland

1. Disease Resistance

Clark - rpm rps1 rps2 rsv1 Rxp (Hm linked with rps1; rj2 with rps2)

Rpm	-L75-6551	C x (Clark 63(6) x Kanrich)
Rpm *	L70-4170	L12 x (Clark 63(5) x Kanrich)
Rpm *	-L76-1169	L6 x (Clark 63(6) x Kanrich)
Rps1	L61-4222(L7)	C(8) x Blackhawk
Rps1	L66-180	PI 101.404A x C(6)
Rps1-b (not hm)	-L77-2061	C(6) x Harrel, Rps1-b hm
Rps1-c	-L75-3901	C(6) x Arksoy
Rps1-c	-L79-1380	C(6) x Higan
Rps1-c rxp	-L76-2107	L6(6) x PI 229.342
Rps1-k	-L77-2015	C(6) x Kingwa
Rps2 (Rj2 rj2)	-L76-2060	C(6) x (H(5) x D54-2437, Rps2 Rj2)#
Rsv1 *	-L78-434	L6(6) x PI 96.983
rxp	L61-4180(L8)	C(8) x CNS
Rps1 rxp	L61-5448(L6)	L8, rxp x L7, Rps1
Rps1 rxp	L60-246(Clark 63)	(C(7) x CNS)rxp x (C(6) x Blackhawk)Rps1

#D54-2437 parentage is Roanoke, Ogden, CNS(source of Rps2), Lincoln, and Richland

2. Nutrient Response

Clark - Fe np Rj1 rj2 rj4

fe	L65-1255(L19)	C(6) x PI 54.619
Np	L63-1677(L9)	C(6) x Chief
Np *	L64-2709	L6 x (C(5) x Chief)
rj1	L63-1889(L13)	C(6) x T201
rj1 *	-L81-4858	L6(2) x L63-1889
Rj2 Rps2 *	-BARC-4	Clark 63(9) x Hardee
Rj4 *	-BARC-2	Clark 63(9) x Hill

3. Stem Growth

Clark - Dt1 dt2 F s

dt1	-L65-792	C(6) x PI 83.945-4
dt1	L63-3297	C(6) x PI 84.987
dt1 *	L72-1737	L6 x L63-3297
dt1	L63-3016	C(6) x PI 86.024
Dt2	L62-1251	C(6) x T117
Dt2 *	L73-811	L6 x L62-1251, Dt2
f	L65-763	C(6) x PI 83.945-4
S	L67-592	C(6) x Higan
S *	L72-1663	L6 x (C(6)x Higan)
s-t *	-L74-373	L12 x (C(6) x Chief)
dt1 Dt2	L73-879	L62-1251, Dt2 x L63-3016, dt1
dt1 S	L72-1745	L67-592, S x "
dt1 s-t	L67-3207	(C(6) x Chief)s-t x L63-3297, dt1
Dt2 S	L71-1284	L67-592, S x L62-1251, Dt2
Dt2 S *	L79-1800(for L74-387)	L6 x (L67-592, S x L62-1251, Dt2)
Dt2 s-t	L67-3224	(C(6) x Chief)s-t x L62-1251, Dt2

* Also Rps1 rxp

Clark Isolines - p. 2

4. Time of Maturity

Clark = e1 E2 E3 (T linked with e1)

E1	L67-1474	C(6) x T175
E1 t	L65-3366	"
E1 t *	L72-1630	L6 x L65-3366, E1 t
e2	L62-1932, L63-3117	C(6) x PI 86.024
e2 *	-L81-4659	L6(6) x Calland
e2 *	L72-1495	L6 x L63-3117, e2
e2 I r *	-L73-695	L12(6) x Chippewa
e2 I r *	-L73-681	L12(6) x Harosoy
e2 I r *	L73-760	L12(6) x Hawkeye
e3	L63-2404	C(6) x PI 84.987
E1 t e2	L66-432	L62-1932, e2 x L65-3366, E1 t
E1 t e3	-L74-441	L63-2404, e3 x "
e2 e3	L71-920	L63-3117, e2 x L63-2404, e3
E1 t e2 e3	-L80-5914	L70-4478, dt1 E1 t e2* x L71-920, e2 e3

4a. Combinations of Stem and Maturity Genes

dt1 E1 t	L66-546	L64-1477, dt1 e2 x L65-3366, E1 t
dt1 e2	L65-778(for L64-1477)	C(6) x PI 86.024
dt1 e3	-L63-3270	C(6) x PI 84.987
dt1 E1 t e2	L66-531(for L66-503)	L64-1477, dt1 e2 x L65-3366, E1 t
dt1 E1 t e2 *	L70-4478	L12 x L66-503, dt1 E1 t e2
dt1 E1 t e3	-L76-865	L63-2404, e3 x L66-546, dt1 E1 t
dt1 E1 t e2 e3	-L80-5879	L70-4478, dt1 E1 t e2* x L71-920, e2 e3
dt1 e2 e3 rxp?	-L80-5882	"
Dt2 e2	L67-3232	L62-1932, e2 x L62-1251, Dt2
Dt2 E1 t	L73-980	L62-1251, Dt2 x L66-432, E1 t e2
Dt2 E1 t e2	L71-1363	"
E1 t S	L71-1388	L67-592, S x "
e2 S	L71-1374	"
E1 t e2 S	L71-1378	"
E1 t s-t	L72-1893	L65-3366, E1 t x L64-1731, s-t Np
e2 s-t	L67-3243(for L67-3246)	L62-1932, e2 x [L6 x (C(5) x Chief)] s-t
e2 s-t *	-L74-434	L6 x L67-3246, e2 s-t
E1 t e2 s-t	L72-1832	L66-531, dt1 E1 t e2 x L67-3246, e2 s-t
dt1 E1 t s-t	L72-1903	" x L67-3207, dt1 s-t
dt1 e2 s-t	L73-904	" x L67-3246, e2 s-t
dt1 E1 t e2 s-t	L71-1403	"

Clark Isolines - p. 3

5. Leaf Form

Clark - Ab Lb1 lb2 lf1 Lf2 Ln Lo Lw1 lw2 T

ab *	L73-1018	L6(6) x Kingwa
ab *	-L75-6697	L6(6) x PI 47.131
lb1	L65-701	C(6) x PI 196.166
lb1 Lb2	-L77-2654	L65-701, lb1(6) x PI 196.166
Lf1	L64-1344	C(6) x PI 86.024
Lf1 *	L72-2157	L12 x L64-1344, Lf1
lf2 *	L73-1087	L6(6) x T255
ln	L62-1579	C(6) x T204
ln *	L70-4629	L12 x L62-1579, ln
lo	L62-1615	C(6) x T204
lw1	L67-1749	C(6) x T176
lw1 t	L65-600	"
Lf1 ln	L64-1083	(C(6) x PI 86.024)Lf1 x (C(6) x T204)ln
ln lo	L70-4313	(C(6) x T204)ln x L62-1615, lo
lw1 Lw2 t	-L79-1685	L65-600, lw1 t(6) x T117, Lw2

6. Pubescence Type

Clark - pl P2 Pal Pa2 pb Pc pd1 pd2 ps

P1	L62-1385	C(6) x T145
p2 I r *	L70-4049	L12(6) x T31
pal pa2	L67-497	C(6) x Higan
pal pa2	-L81-4651	L70-4558, pa2 x L76-1291, pal
pal	-L76-1291	C(6) x Higan
pa2	L70-4558	"
Pb *	L73-1034	L6(6) x Kingwa
pc	L63-2435	C(6) x PI 84.987
Pd1	L62-1686	C(6) x PI 80.837
Pd1 *	L73-1046	L12 x L62-1686, Pd1
Pd2 *	-L75-6648	L6(6) x T264
Ps	L63-2999	C(6) x PI 91.160
Ps *	L71-149	L12 x L63-2999, Ps
Ps-s	L64-314	C(6) x Higan
P1 pc	L67-3124	(C(6) x PI 84.987)pc x L62-1385, P1
P1 Pd1	L67-3770	L62-1385, P1 x (C(6) x PI 80.837)Pd1
P1 Ps	L67-3127	" x (C(6) x PI 91.160)Ps
pal pa2 pc	L73-1101	L67-497, pal pa2 x L63-2435, pc
pal pa2 Pd1	L73-1118	" x L62-1686, Pd1
pal pa2 Ps	L73-1144	" x L63-2999, Ps
pal pa2 Ps-s	L67-495	C(6) x Higan
pal Ps-s	L64-326	"
pa2 Ps-s	L70-4566	"
pc Pd1	L65-44	(C(6) x PI 84.987)pc x (C(6) x PI 80.837)Pd1
pc Ps	L65-52	" x (C(6) x PI 91.160)Ps
pc Ps-s	L68-1864	(C(6) x Higan)Ps-s x L63-2435, pc
Pd1 Pd2 rxp?	-L79-1815	L62-1686, Pd1 x (L6(6) x T264)Pd2
Pd1 Ps	L65-90	(C(6) x PI 80.837)Pd1 x (C(6) x PI 91.160)Ps
Pd1 Ps-s	L68-1874	(C(6) x Higan)Ps-s x (C(6) x PI 80.837)Pd1

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7. Chlorophyll

Clark = cyt-Y D1 D2 g V1 Y3 Y7 Y8 Y9 Y11

cyt-G	L62-1027	Medium Green x C(7)
d1 d2	L69-4663(for L67-1000)	C(6) x Columbia
d2	L69-4662	"
G d1 d2	L64-2545	"
G d1	L69-4659	"
G d2	L69-4667(for L69-4666)	"
v1 *	-L76-1149	L6(6) x T93
y3	L63-2346	C(6) x T139
y7 y8	L63-1792	C(6) x T138
y9 *	L69-4755	L6(6) x T135
Y11 y11	L72-1937(for L65-1237)	C(6) x T219H
cyt-G y3	L64-2584	L62-1027, cyt-G x (C(6) x T139)y3
cyt-G y9 **	-L74-824	" x L69-4755, y9*
cyt-G Y11 y11	-L74-826	" x L65-1237, Y11 y11
d1 d2 y9 **	-L74-836	(C(6) x Columbia)G d1 d2 x L69-4755, y9*
G d1 d2 Y11 y11	-L74-838	" x L65-1237, Y11 y11
d1 d2 y3	-L74-851	L67-1000, d1 d2 x L63-2346, y3
d1 d2 y7 y8	-L74-852	L69-4663, d1 d2 x L63-1792, y7 y8

8. Pigmentation

Clark = fl i-i im K1 K2 K3 11 L2 M1 O R T Td W1 w3 W4 Wm

Fl i *	L73-1004	L6 x (L67-3472, i*(5) x PI 47.131)
I	L62-1058	C(6) x T201
i(wild)	-L68-236	PI 101.404A x C(6)
i	L67-3469	mutation in Clark found in 1954 at Urbana
i *	L67-3472	mutation in Clark 63 found in 1965 Illinois foundation seeds
i *	L66-14	mutation in Clark 63 received in 1965 from Portageville, MO
i-k *	L70-4204(for L70-4209), L66-14, i*(6)	x Black Eyebrow
	L69-4607	
Im I r *	L69-5338, -L69-5343	L12(6) x Hawkeye
Im r *	L69-5366	"
k1	L67-3479	mutation in Clark from Ames, Iowa in 1956
k1 *	L67-3480	mutation found in 1965 Illinois foundation seeds of Clark 63
k2	L67-3483(S57-3491)	mutation in x-rayed Clark at Columbia, MO
k3	T238	mutation in x-rayed Clark found in 1956 at Columbia, Missouri
L1 *	-L70-4413	L6(6) x (Laredo x Harosoy)
L1 *	L68-1562	L6(6) x Seneca
L2	L68-1013	C(6) x Higan
mi G t *	-L76-1010	L6 x [L68-2063, r t w*(5) x (Laredo x Harosoy)]
o i r *	L72-2004	L67-3484, i r*(6) x Ogemaw
r	-L65-1068	C(6) x Higan
r	L62-1383	C(6) x T145
r *	L65-1914	L6 x L11, I r
i r-m *	L72-2040	L67-3484, i r*(6) x PI 91.073
t	L67-483	C(6) x Higan
t	-L65-601	C(6) x T176
t	-L85-1467	C(6) x PI 84.987

Clark Isolines - p. 5

8. Pigmentation (continued)

td	L66-260	C(6) x PI 91.160
td *	L66-228	L6(6) x Sooty
td *	L70-4404	L6(6) x Grant
w1	L63-2373	C(6) x T139
w1 *	L69-4776	L6(6) x Seneca
w1	-L65-1077	C(6) x PI 83.945-4
W3 w4 *	L70-4422	L6(6) x (Laredo x Harosoy)
w4 *	L68-1774	"
w4 *	-L81-4945	L6(6) x PI 81.763
wm *	L72-2181	L6(6) x T235
I r	L64-2191(L11)	(C(6) x T201)I x (C(6) x T145)r
I r *	L64-2244(L12)	L6 x L11, I r
I t	-L86-1084	L12 x L64-2281 sib, t w1
I t *	-L83-879, L86-1078	"
I w1 **	-L84-1231	"
I r w1 Rps1 rps1	-L81-5080	"
I t w1	-L86-1098	"
I t w1 **	-L83-841, L86-1096	"
I r t *	L70-4543	L12(6) x Hawkeye
I r t w1 *	L68-2056	L12 x L64-2281 sib, t w1
i r	L66-17	i-mutation in L11, I r found in 1965 at Urbana
i r *	L67-3484	L66-14, i* x L12, I r*
i t	L68-2073	L67-3469, i x L64-2281 sib, t w1
i w1	L68-2077	"
i t w1	L70-4497	"
i r t *	-L83-930	L66-14, i* x (L12 x L64-2281 sib, t w1)
i r w1 *	-L83-942	"
i r t w1 *	-L82-2669	"
i-k t *	-L76-1101	L68-2063, r t w1* x L70-4209, i-k*
i-k w1 *	-L76-1113	"
i-k r o *	L74-1089	L69-4607, i-k* x (L67-3484, i r*(5) x Ogemaw, o)
i-k r t *	-L75-6426	L68-2063, r t w1* x L70-4209, i-k*
i-k r w1 *	-L75-6433	"
i-k t w1 *	-L76-1122	"
i-k r t w1 *	-L75-6439	"
k1 t1	L68-2082	L67-3479, k1 x L64-2281 sib, t w1
k1 w1	L68-2085	"
k1 t w1	L68-2093	"
k1 r	L68-2106	" x L12, I r*
I k1	L68-2130	"
I k1 r	L68-2105	"
12 t	L68-1017	C(6) x Higan
12 w1	L69-4814	C(6) x T204
r t	-L86-1092	L12 x L64-2281 sib, t w1
r t **	-L83-900	"
r w1 *	L68-2061, L81-5082	"
r t w1 *	L68-2063	"
t w1	L64-2281	(C(6) x T139)w1 x (C(6) x T204)t
t td	L73-1071	L67-483, t x L66-228, td*
t w4 *	-L84-1135	L6(6) x (Laredo x Harosoy)
td w1 *	L69-4775	L6(6) x Seneca
wm t *	L72-2210	L6(6) x T235

Clark Isolines - p. 6

9. Other

Clark - *bl Hb Msl Ms2 N Ti-a*

<i>B1 i</i>	L69-4544	L67-3469, i(6) x Sooty
<i>hb *</i>	-L78-3263	L6(6) x PI 229.342
<i>msl *</i>	L74-03	L6(6) x T260H
<i>ms2 *</i>	-L79-1308	L6(6) x T259H
<i>n *</i>	L72-1987	L6(6) x Soysota
<i>ti *</i>	-L81-4871	L6(6) x PI 157.440
semi-wild cytoplasm	L68-1306	PI 65.388 x C(6)
wild cytoplasm	L66-183	PI 101.404A x C(6)
wild cytoplasm	L67-1189	PI 101.404B x C(6)
chromosome	-L74-1060(A72-1036)	"
translocation		
heterozygous for	-L70-9283	"
translocation		

10. Combinations transferred together (linked genes?)

<i>dtl e2 Lf1</i>	L65-774	C(6) x PI 86.024
<i>dtl Lf1</i>	L63-3022	"
<i>dtl Rps1-b hm</i>	-L77-2050	C(6) x Harrel
<i>e2 Im *</i>	L72-1582	L6 x (L12(5) x Hawkeye)
<i>e2 Lf1</i>	L64-1316	C(6) x PI 86.024
<i>e2 12 Rps1-c rxp</i>	-L76-2096	L6(2) x (L63-3117, e2(4) x PI 229.342)
<i>e2 t *</i>	L72-1568	L6(6) x T235
<i>e2 wm *</i>	L72-1566	"
<i>e3 pc</i>	-L63-2441	C(6) x PI 84.987
<i>i Rps1</i>	-L68-237	PI 101.404A x C(6)
<i>12 Rps1-c</i>	-L79-1404	C(6) x Arksoy
<i>12 Rps1-c rxp</i>	-L76-2100	L6(6) x PI 229.342
<i>ln t</i>	L62-1568	C(6) x T204
<i>n r *</i>	L72-1977	L6(6) x Soysota
<i>Np s-t</i>	L64-1731	C(6) x Chief
<i>Pl r</i>	L62-1377	C(6) x T145
<i>pal pa2 S</i>	L67-509	C(6) x Higan

Also *E1 t*, *dtl e2*, *dtl e3*, *G d1*, *12 t*, *12 w1*, *pal Ps-s*, *t w4*, *td w1*, and *wm t* in other sections. Combinations transferred together from one donor by selection are *d1 d2*, *G d2*, *lwl t*, *pal pa2*, *pa2 Ps-s*, *W3 w4*, and *y7 y8*, listed in other sections.

Clark Isolines - p. 7

11. Miscellaneous recombinations

d1 d2 p2 *	L72-2824	(L12(5) x T31)p2* x (C(6) x Columbia)d1 d2
d1 d2 p2 I r *	L72-2832	"
dt1 El t e2 I r *	L73-944	(L12(6) x Hawkeye)Im* x L66-531,dt1 El t e2
dt1 El t e2 Im *	L72-1853	"
dt1 El t e2 Pdl	L72-1885	L66-531,dt1 El t e2 x L62-1686,Pdl
dt1 i r *	-L73-671	L67-3484,i r*(6) x PI 91.073
dt1 Ll	L72-1727	(L6(6) x Seneca)Ll* x L63-3297,dt1
dt1 Ll	-L83-964	L68-1562,Ll* x L63-3016,dt1
dt1 ln	-L71U-659	L63-3297,dt1 x L62-1579,ln
dt1 ln Rps1	-L72U-4282	L62-1579,ln x (L63-3297,dt1 x Clark 63)
dt1 ln Pdl	-L73U-2774	(L63-3297,dt1 x L62-1579,ln) x
		(L63-3297,dt1 x L62-1686,Pdl)
dt1 Pdl	-L72U-4304	L63-3297,dt1 x L62-1686,Pdl
dt1 Pdl *	-L72U-4317	Clark 63 x (L63-3297,dt1 x L62-1686,Pdl)
Dt2 Lf1	L64-1081	(C(6) x T117)Dt2 x (C(6) x PI 86.024)Lf1
Dt2 Lf1 ln	L68-2040	L64-1074,Dt2 ln x "
Dt2 ln	L64-1074	(C(6) x T117)Dt2 x (C(6) x T204)ln
El t e2 Pdl	L72-1879	L66-531,dt1 El t e2 x L62-1686,Pdl
e2 hb *	-L75-6631	L72-1495,e2*(6) x PI 229.342
e2 I Im r *	L73-753	L12(6) x Hawkeye,e2 Im*
I Pl r	L68-2045	L62-1058,I x (C(6) x T145)Pl r
I r rjl *	L72-2133	L12 x L63-1889,rjl
I r Rpm *	L70-4190(for L70-4191)	L12 x (Clark 63(5) x Kanrich)
i ti *	-L83-693	i mutation in L6(6) x PI 157.440
ln I r *	L72-2111	L12 x L62-1579,ln
ln Pdl	-L72U-4191	L62-1579,ln x L62-1686,Pdl
ln Pdl Rps1	-L74U-6710	" x (Clark 63 x L62-1686,Pdl)
lo I r *	L70-4611	L12 x L62-1615,lo
r rjl *	L73-1054	" x L63-1889,rjl
r Rpm *	-L70-4186	" x (Clark 63(5) x Kanrich)

GENE(S) LINE PARENTAGE

HAROSOY ISOLINES

- L58-266(Harosoy) Mandarin (Ottawa)(2) x AK (Harrow)

1. Disease Resistance

Harosoy = *rmd rps1 rps2 rps5 Rxp* (*Hm* linked with *rps1*; *rj2* with *rmd rps2*)

<i>Rps1</i>	L59-731(Harosoy 63)	H(8) x Blackhawk
<i>Rps1-c hm?</i>	-L85-129	H(6) x Higan
<i>Rps2 Rmd Rj2</i>	-L82-1449	H(6) x D54-2437 #
<i>Rps5 w1</i>	-L62-904	H(6) x PI 91.160
<i>rxp</i>	L61-4094(L3)	H(6) x S54-1207 #
<i>Rps1 rxp</i>	L61-5047(L2)	Harosoy 63 x L3, <i>rxp</i>
<i>Rps1 rxp</i>	L68-758	H(4) x L2, <i>Rps1 rxp</i>
<i>Rps1 Rps2 Rmd Rj2</i>	-L86-493	L2, <i>Rps1</i> x (H(5) x D54-2437) <i>Rps2 Rmd Rj2</i> #

D54-2437 parentage is Roanoke, Ogden, CNS, Lincoln, and Richland; *Rps2* is presumably from CNS.

S54-1207 parentage is Hawkeye x ((Lincoln(2) x Richland) x ((Lincoln(2) x Richland) x (Lincoln x CNS))); *rxp* is from CNS.

2. Nutrient Response

Harosoy = *Fe np Rj1* (for *rj2* see above section)

<i>fe</i>	L66-731(L18)	H(6) x PI 54.619
<i>Np</i>	L66-704(L17)	H(6) x (C(6) x Chief)
<i>rj1</i>	L65-1274(L14)	H(6) x T201
<i>rj1 *</i>	L66-2470	L2 x (H(6) x T201)

3. Stem Growth

Harosoy = *Dt1 dt2 F Mn s*

<i>dt1</i>	L67-153	H(6) x Higan
<i>dt1</i>	L62-973	H(6) x PI 86.024
<i>dt1 *</i>	L72-1177	L2 x L62-535, <i>dt1</i> (H(6) x T145)
<i>Dt2</i>	L62-364	H(6) x T117
<i>Dt2 *</i>	L67-1250	L2 x (H(6) x T117)
<i>Dt2</i>	L63-1397	H(6) x PI 80.837
<i>f</i>	L65-756	H(6) x PI 83.945-4
<i>mn</i>	T251H	mutation in Harosoy(5) x T139 found in 1961 at Urbana
<i>S</i>	L67-234	H(6) x Higan
<i>S *</i>	L72-1198	L2 x L67-234, <i>S</i>
<i>dt1 Dt2</i>	L67-3256	(H(6) x T117) <i>Dt2</i> x L62-973, <i>dt1</i>
<i>dt1 S</i>	L72-1228	L67-234, <i>S</i> x L67-153, <i>dt1</i>
<i>Dt2 S</i>	L72-1241	L67-234, <i>S</i> x L63-1397, <i>Dt2</i>
<i>Dt2 S *</i>	L73-105	(L2 x L67-234) <i>S*</i> x L67-1250, <i>Dt2*</i>

* Also *Rps1 rxp*

Harosoy Isolines - p. 2

4. Time of Maturity

Harosoy - e1 e2 E3 e5 (t linked with e1)

E1	-L64-4103	H(6) x Columbia
E1	L68-694	H(6) x PI 196.166
E1 T	L67-2324	"
E1 T *	L72-1304	L2 x L67-2324, E1 T
E2	-L64-4584	H(6) x T117
E2 *	-L74-27	L2(6) x Clark
e3	L62-667(for L73-1543)	H(6) x T204
E5	-L64-4830	H(6) x PI 80.837
E1 T E2	-L71L-3004	L67-2324, E1 T x L64-4584, E2
E1 T e3	L71-802	L62-667, e3 x L67-2324, E1 T
E1 T E5	-L71L-3015	L67-2324, E1 T x L64-4830, E5
E2 e3	-L84-307	L64-4584, E2 x L73-1543, e3
E2 E5	-L74-66	" x L64-4830, E5
E2 E5	-L86L-4	L64-4830, E5 x L74-27, E2*
e3 E5	-L84-337	" x L73-1543, e3

4a. Combinations of Stem and Maturity Genes

dt1 E1 T	L71-1116	L67-2324, E1 T x L67-153, dt1
Dt2 E1 T	L74-59	" x L67-1397, Dt2
Dt2 E5	-L62-812	H(6) x PI 80.837
E1 T S	L71-1106	L67-234, S x L67-2324, E1 T
Dt2 E1 T S	-L79-842	L71-1106, E1 T S x L74-59, Dt2 E1 T

5. Leaf Form

Harosoy - Ab lf1 Ln Lo Lw1 (lw2)

ab *	L73-67	L2(6) x Kingwa
Lf1	L62-956	H(6) x PI 86.024
ln	L63-1212	H(6) x T204
lo	L65-372	"
lw1	L65-461	H(6) x T176
Lf1 ln	L64-1069	(H(6) x PI 86.024)Lf1 x (H(6) x T204)ln
ln lo	L70-4136	(H(6) x T204)ln x (H(6) x T204)lo

6. Pubescence Type

Harosoy - p1 P2 Pal Pa2 pb Pc pd1 ps

P1	L62-561	H(6) x T145
p2 *	L70-4001	L2(6) x T31
pal	L67-271	H(6) x Higan
pa2	L70-4112(for L70-4119)	"
pal pa2	L69-6095	"
pal pa2	-L81-4075	L67-271, pal x L70-4112, pa2
Pb *	L73-79	L2(6) x Kingwa
pc (Fg3, SGN 12:32)	L63-1097	H(6) x PI 84.987
Pd1	L62-801	H(6) x PI 80.837
Pd1 *	L71-46	L2 x L62-801, Pd1
Ps	L62-880	H(6) x PI 91.160
Ps-s	L67-166	H(6) x Higan
P1 pc	L67-3099	(H(6) x PI 84.987)pc x L62-561, P1
P1 Pd1	L67-3101	(H(6) x PI 80.837)Pd1 x "
P1 Ps	L67-3104	(H(6) x PI 91.160)Ps x "
p1 Ps-s	L65-237	H(6) x Higan
pc Pd1	L65-25	(H(6) x PI 84.987)pc x (H(6) x PI 80.837)Pd1
pc Ps	L65-34	" x (H(6) x PI 91.160)Ps
Pd1 Ps	L65-60	(H(6) x PI 91.160)Ps x (H(6) x PI 80.837)Pd1

Harosoy Isolines - p. 3

7. Chlorophyll

Harosoy - cyt-Y D1 D2 g Y3 Y7 Y8 Y9 Y20			
cyt-G	L62-17	Medium Green x H(7)	
d1 d2	L69-4267	H(6) x Columbia	
d1	L73-54	L69-4268,G d1 x L69-4267,d1 d2	
d2	L69-4266	H(6) x Columbia	
G d1 d2	L64-2489	"	
G d1	L69-4265(for L69-4268)	"	
G d2	L67-971	"	
y3	L63-1016	H(6) x T139	
y7 y8	L68-560	H(6) x T138	
y9 *	L69-4318	L2(6) x T135	
y20 k2	T253	mutation in T239(k2) found in 1963 at Urbana	

8. Pigmentation

Harosoy - I K2 l1 L2 r t W1 w3 W4 Wm			
i-i	L67-38	H(6) x Clark	
i-i	-L67-949	H(6) x Columbia	
i-i	-L65-1053	H(6) x T117	
i-i	-L65-556	H(6) x T176	
i	L67-3388	mutation in Harosoy found in 1959 at Urbana	
i *	L67-3396	mutation in L2 found in 1963 at Shabbona, IL	
k2	T239	mutation in Harosoy found in 1961 at Urbana	
L1 *	L68-582	L2(6) x Seneca	
L1 *	-L69-4428	L2(6) x PI 81.763	
l2	L67-226	H(6) x Higan	
l2	-L62-542	H(6) x T145	
R	-L63-1069	H(6) x T139	
R	L65-540	H(6) x T176	
T	L66-707	H(6) x Clark	
w1	-L64-2139	H(6) x Peking	
w1	L62-906	H(6) x PI 91.160	
W3 w4 *	L72-1078	L2(6) x Laredo	
w4 *	L72-1138	"	
wm	T235	mutation in Harosoy found in 1957 at Urbana	
i-i R	L67-1695	H(6) x T176	

Harosoy Isolines - p. 4

9. Other

Harosoy - Msl N		
msl (Tonica)	T267H	mutation in Harosoy found in 1955 at Tonica, Illinois
n *	L72-1140	L2(6) x Soysota

10. Combinations transferred together (linked genes?)

Dt2 i-i	L65-1058	H(6) x T117
E2 T *	-L74-21	L2(6) x Clark
i-i Np	L66-721	H(6) x L9(Clark-Np)
i-i R lw1	L67-1687	H(6) x T176
12 P1	L62-558	H(6) x T145
12 Rps1-c hm?	-L85-144	H(6) x Higan
12 S	L67-225	"
Np T	L66-713	H(6) x L9(Clark-Np)

Also Dt2 E2, Dt2 E5, E1 T, i-i R, G d1, pal Ps-s, Rps5 w1, Rps1-c hm, Rps2 Rmd Rj2 in other sections. Combinations transferred together from one donor by selection are d1 d2, pal pa2, W3 w4, and y7 y8, listed in other sections.

11. Miscellaneous recombination

d1 d2 p2 *	L70-4037	(L2(5) x T31)p2* x (H(6) x Columbia)d1 d2
dt1 ln	-L72D-4110	L63-1212,ln x L62-535,dt1 (H(6) x T145) #
dt1 ln Rps1	-L73D-2743	(L63-1212,ln x Harosoy 63) x (L62-535,dt1 x Harosoy 63) #
dt1 Pd1	-L73D-2733	L62-535,dt1 x L62-801,Pd1 #
dt1 ln Pd1	-L72D-4169	(L62-535,dt1 x L62-801,Pd1) x (L62-535,dt1 x L63-1212,ln) #
Dt2 Lf1 ln	L67-3298	[(H(6) x T117)Dt2 x (H(6) x T204)ln] x [(H(6) x T117)Dt2 x (H(6) x PI 86.024)Lf1]
ln Pd1	-L72D-4028	L63-1212,ln x L62-801,Pd1
ln Pd1 Rps1	-L72D-4029	(L63-1212,ln x Harosoy 63) x L62-801,Pd1
ln Rps1	-L72D-4045	L63-1212,ln x Harosoy 63
wm Rps1	L63-1612	Harosoy 63 x [(T235(2) x H(5) x Blackhawk)]

L62-535 is not maintained, see L72-1177,dt1

Miscellaneous Isolines - p. 1

<u>GENE(S)</u>	<u>LINE</u>	<u>PARENTAGE</u>
AMSOY 71 ISOLINES		
<i>dtl</i>	-L82-1011	Amsoy 71(6) x PI 151.440
<i>ti</i>	L83-4387	"

BEESON ISOLINE

<i>ms2</i>	-L75-0587	Beeson(6) x T259
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CHIPPEWA ISOLINES

Chippewa (Ch) - e2 i-i im p1 R rpm rps1 Rxp T W1

<i>Rps1</i>	Chippewa 64	Ch(8) x Blackhawk
<i>Rps1</i>	L63-16	Ch(10) x Blackhawk
<i>rxp</i>	L63-42	Ch(8) x (C1128(2) x S54-1207) †
<i>Rps1 rxp</i>	L64-2721(L10)	(Ch(8) x CNS) x (Ch(10 x Blackhawk
<i>I r *</i>	L66-892(L16)	[L10(6) x (Clark(6) x T201)]I* x
		[L10(6) x (Clark(6) x T145)]P1 r*
<i>I P1 r *</i>	L67-3586	same as above
<i>Rpm *</i>	L68-4172(SL7)	L10 x [Chippewa 64(5) x (Clark(2) x Kanrich)]
<i>I r Rpm *</i>	L68-4216(SL8)	L16 x SL7
<i>I t w1 Rpm *</i>	L68-4242	L10 x (Chippewa 64(4) x S62X30:1) #
<i>E2 *</i>	L68-4291	L10(6) x Clark
<i>Im Rpm *</i>	-L72-607	SL7 x [L16 x (L10(2) x Merit)]

* = also *Rps1 rxp* from L10† = C1128 is from Wabash x Hawkeye. S54-1207 has *rxp* from CNS. See Harosoy section 1.# The backcrosses to Clark were done at the University of Missouri by L.F. Williams. S62X2 is from [Clark(2) x (Lincoln(2) x Richland)] *I t w1* x (Clark 63(3) x Kanrich) *Rpm Rps1 rxp*.

CORSOY ISOLINES

Corsoy - *rps1 rps2 rj2*

<i>Rps1-c</i>	Corsoy 79	Corsoy(6) x Lee 68
<i>Rps1-k</i>	-L78-189(L27)	Corsoy(8) x Kingwa
<i>Rps2 Rj2</i>	-L77-1585	Corsoy(6) x (Harosoy(5) x D54-2437) #
<i>Rps1-c Rps2 Rj2</i>	-L80-5015(L28)	Corsoy 79 x L77-1585, <i>Rps2 Rj2</i>
<i>Rps1-k Rps2 Rj2</i>	-L82-1187	L77-1585, <i>Rps2 Rj2</i> x (Corsoy(8) x Kingwa) <i>Rps1-</i>

D54-2437 parentage is Roanoke, Ogden, CNS(source of *Rps2 Rj2*), Lincoln, and Richland

ELF ISOLINE

<i>Dtl</i>	-L81-4274	Elf(6) x Williams
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Miscellaneous Isolines - p. 2

WAYNE ISOLINES

Wayne - E2 i-i im Ln pl R rpm rpsl

Rpsl	L65-4059(L15)	W(6) x Clark 63
I r	L66-949(L20)	W(5) x L68-2045,Clark-I Pl r
I Pl r	L67-3521(for L67-3522)	"
I r Rpsl	L67-3542(for L67-3526)	L15 x (W(4) x L68-2045,Clark-I Pl r)
Rpm	L68-4064(SL9)	W(10) x Kanrich
Rpm Rpsl	L69-4124(SL10)	L15,Rpsl x SL9,Rpm
I r *	L69-4180(SL12)	L67-3526,I r Rpsl x SL9,Rpm
ln I r *	L72-1369	SL12(6) x (Clark(6) x T204)ln
e2 I r *	L72-1404(for L72-1392)	SL12(6) x (Clark(6) x PI 86.024)e2
I r *	L72-1424	L15,Rpsl x SL12,I r *
Im I r *	L73-210,L73-212	SL12(6) x Merit
e2 ln I r *	-L74-142	L72-1369,ln I r * x L72-1392,e2 I r *
e2 ln I r Rpsl	-L74-143	"
e2 I r Rpsl	-L76-612, -L77-2152	L15 x (SL12(5) x Merit)

* also Rpm Rpsl

WELLS ISOLINE

ms2	-L75-0570	Wells(6) x T259
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PI 65.549 ISOLINE (wild soybean)

Rsvl	-L83-0214,-L83-0215	PI 65.549(6) x PI 96.983
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GENE(S)LINEPARENTAGE

WILLIAMS ISOLINES

1. Disease Resistance

Williams = *Rmd rpm rpp1 rpp2 rpp4 rps1 rps2 rps3 rps4 rps5 rpv rsv1 rsv2*
(*Hm* linked with *rps1*; *rj2* with *rps2*)

<i>rmd</i>	-L84-2237	Wm(6) x (Clark 63(2) x PI 86.150)
<i>rmd</i>	-L88-8226	Wm(6) x PI 86.972-1
<i>Rpm</i>	-L80-5427	Wm x L75-6125, <i>Rpm Rps1</i> ; = Wm(2) x Union
<i>Rpp1 Rps1-k</i>	-L85-2378	Wm 82(6) x PI 200.492
<i>Rpp2 Rps1-k</i>	-L86-1752	Wm 82(6) x PI 230.970
<i>Rpp4 Rps1-k</i>	-L87-0482	Wm 82(6) x PI 459.025
<i>Rps1-b hm</i>	-L77-1863(for L77-1818)	Wm(7) x Harrel
	(L26)	
<i>Rps1-c</i>	L75-3735 (Wm 79)	Wm(6) x Lee 68
<i>Rps1-c</i>	-L77-1727	Wm(6) x (Clark 63(3) PI 229.342)
<i>Rps1-k</i>	L77-1794 (Wm 82)	Wm(7) x Kingwa
<i>Rps2 Rj2</i>	-L76-1988(for L76-2013)	Wm(6) x (Harosey(5) x D54-2437) #
<i>Rps3</i>	-L83-570	Wm(6) x PI 86.972-1
<i>Rps4</i>	-L85-2352	Wm(6) x PI 86.050
<i>Rps5</i>	-L85-3059	Wm(6) x PI 91.160
<i>Rpv</i>	-L85-2308	Wm(6) x Dorman
<i>Rsv1</i>	-L78-379 (L25)	Wm(6) x PI 96.983
<i>Rsv2</i>	-L88-8431	Wm(6) x Raiden, PI 360.844

D54-2437 parentage is Roanoke, Ogden, CNS, Lincoln, and Richland. *Rps2 Rj2* is from CNS

1a. Disease Resistance Combinations

<i>Rps1-c Rps2 Rj2</i>	-L81-4352	Wm 79, <i>Rps1-c</i> x L76-2013, <i>Rps2 Rj2</i>
<i>Rpm Rps1</i>	-L75-6141(for L75-6125)	Wm(6) x SL12, Wayne- <i>Rpm Rps1</i> ; = Wm x Union
<i>Rpm Rps1-b hm</i>	-L88-8502	L75-6125, <i>Rpm Rps1</i> x L77-1818, <i>Rps1-b hm</i>
<i>Rpm Rps1-c Rps2 Rj2</i>	-L82-1858	L81-4352, <i>Rps1-c Rps2 Rj2</i> x (L75-6141, <i>Rpm Rps1</i> x L76-1988, <i>Rps2 Rj2</i>)
<i>Rpm Rps1-k</i>	-L88-8488(for L80-5493)	Wm 82, <i>Rps1-k</i> x (Wm x L75-6125, <i>Rpm Rps1</i>)
<i>Rpm Rsv1</i>	-L83-4744	L78-379, <i>Rsv1</i> x L80-5493, <i>Rpm Rps1-k</i>
<i>Rpm Rps1-k Rsv1</i>	-L83-4752	"
<i>Rps1-k Rsv1</i>	-L81-4420	" x Wm 82, <i>Rps1-k</i>

2. Stem Growth

Williams = *Dt1 dt2*

<i>dt1</i>	-L85-2029	Wm(6) x (Clark 63(2) x PI 86.150)
<i>dt1</i>	-L88-8025	Wm(6) x PI 408.251
<i>Dt2</i>	L74-189 (Will)	Wm(6) x (Clark(6) x T117)

Williams Isolines - p. 2

2a. Will Isolines (Will = Williams + Dt2)

Will = Ep im rpm rps1 rsv1

Dt2 ep	-L74-221(Will subline)	Wm(6) x (Clark(6) x T117)
Dt2 Im	-L79-1270	Will x L75-6867, Im
Dt2 Rps1-k	-L79-1250(for L79-1224)	" x Wm 82
Dt2 Rsv1	-L80-5227(for L80-5230)	" x (Wm(6) x PI 96.983)
Dt2 Rpm Rps1	-L81-4308(for L79-1178)	" x L75-6125, Rpm1 Rps1
Dt2 Rpm Rsv1	-L82-753	L79-1178, Dt2 Rpm Rps1 x L80-5227, Dt2 Rsv1
Dt2 Rps1 Rsv1	-L83-4494	"
Dt2 Rpm Rps1 Rsv1	-L83-4483	"
Dt2 Rpm Rps1-k	-L82-1560	" x L79-1224, Dt2 Rps1-k
Dt2 Rps1-k Rsv1	-L83-4527	L79-1224, Dt2 Rps1-k x L80-5227, Dt2 Rsv1
Dt2 Rpm Rps1-k Rsv1		-L88-8739 " x (L79-1178, Dt2 Rpm
Rps1		x L80-5230, Dt2 Rsv1)

3. Pigmentation

Williams = i-i im l2 T w1

I Rps2 Rj2	-L76-1994	Wm(6) x (Harosoy(5) x D54-2437)
i	-L88-5492	mutant found in Williams in 1980 from a farm on Windsor Road, Champaign, IL
i (not Ti-b)	-L84-2003	mutation in Wm(6) x Jefferson found in 1982 at Urbana
i Rps1-c	-L88-5495	mutant found in Williams(6) x Lee 68 in 1975 at Urbana (L75-3774)
Im	-L77-5632(for L75-6867)	Wm(6) x L69-5343, Clark-I r Im*
Im Rps1-c	-L80-5452	Wm 79, Rps1-c x L75-6867, Im
L2	-L78-3083	Wm(6) x L69-5343, Clark-I r Im*
L2 Im	-L78-3079	"
r Rsv2	-L88-8440	Wm(6) x Raiden (PI 360.844)
t Rps2 Rj2	-L76-2023	Wm(6) x (Harosoy(5) x D54-2437)
W1 Rps1-c	-L79-908	Wm(6) x Lee 68
W1 Rps1-c	-L80-5372	Wm(6) x (Clark 63(6) x PI 229.342)

4. Seed Composition

Williams = Lx1 Sun Ti-a

Lx1	-L86-1436	Wm(6) x PI 408.251
sun	-L85-2196	Wm(6) x PI 229.324
ti Rps1-k	-L81-4590(= Kunitz)	Wm 82(6) x PI 157.440
Ti-b	-L82-2020	Wm(6) x Jefferson
Ti-c	-L82-2051	Wm(6) x PI 196.172

5. Other Traits and Combinations

Williams = E2 Ms2

cytoplasm	-L78-3130	Wisconsin Black x Wm(6)
dt1 Ti-c	-L82-2045	Wm(6) x PI 196.172
dt1 i Ti-c	-L88-8012	i mutant selected from Wm(6) x PI 196.172
e2	-L75-6111	Wm(6) x [SL12, Wayne-I r Rpm Rps1(6) x (Clark(6) x PI 86.024)e2]
ms2	L74-01	Wm(6) x T259H
and Ti-b	-L82-2024	Wm(6) x Jefferson

C-1

APPENDIX 1: CLARK ISOLINES ARRANGED BY LINE DESIGNATION

Clark - Ab b1 cyt-Y D1 D2 Dt1 dt2 e1 E2 E3 F Fe f1 g Hb Hm i-i im K1 K2 K3 l1 L2
 Lb1 lb2 lfl Lf2 Ln Lo Lw1 lw2 Mi Msl Ms2 N np O pl P2 Pal Pa2 pb Pc pd1 pd2
 ps R Rj1 rj2 rpm rps1 rps2 rsv1 Rxp s T Td Ti-a V1 W1 w3 W4 Wm Y3 Y7 Y8 Y9 Y11

Rj4 *	BARC-2	Clark 63(9) x Hill
Rj2 Rps2 *	BARC-4	Clark 63(9) x Hardee
-	L58-231(Clark)#	Lincoln(2) x Richland
Rps1 rxp	L60-246(Clark63)	(C(7) x CNS)rxp x (C(6) x Blackhawk)Rps1
rxp	L61-4180(L8)	C(8) x CNS
Rps1	L61-4222(L7)	C(8) x Blackhawk
Rps1 rxp	L61-5448(L6)	L8,rxp x L7,Rps1
cyt-G	L62-1027	Medium Green x C(7)
I	L62-1058	C(6) x T201
Dt2	L62-1251	C(6) x T117
Pl r	L62-1377	C(6) x T145
r	L62-1383	"
Pl	L62-1385	"
ln t	L62-1568	C(6) x T204
ln	L62-1579	"
lo	L62-1615	"
Pd1	L62-1686	C(6) x PI 80.837
e2	L62-1932	C(6) x PI 86.024
Np	L63-1677(L9)	C(6) x Chief
y7 y8	L63-1792	C(6) x T138
rj1	L63-1889(L13)	C(6) x T201
y3	L63-2346	C(6) x T139
w1	L63-2373	"
e3	L63-2404	C(6) x PI 84.987
pc	L63-2435	"
e3 pc	-L63-2441	"
Ps	L63-2999	C(6) x PI 91.160
dt1	L63-3016	C(6) x PI 86.024
dt1 Lf1	L63-3022	"
e2	L63-3117	"
dt1 e3	-L63-3270	C(6) x PI 84.987
dt1	L63-3297	"
Ps-s	L64-314	C(6) x Higan
pal Ps-s	L64-326	"
Dt2 ln	L64-1074	(C(6) x T117)Dt2 x (C(6) x T204)ln
Dt2 Lf1	L64-1081	(C(6) x T117)Dt2 x (C(6) x PI 86.024)Lf1
Lf1 ln	L64-1083	(C(6) x PI 86.024)Lf1 x (C(6) x T204)ln
e2 Lf1	L64-1316	C(6) x PI 86.024
Lf1	L64-1344	"
Np s-t	L64-1731	C(6) x Chief
I r	L64-2191(L11)	(C(6) x T201)I x (C(6) x T145)r
I r *	L64-2244(L12)	L6 x L11,I r
t w1	L64-2281	(C(6) x T139)w1 x (C(6) x T204)t
G d1 d2	L64-2545	C(6) x Columbia
cyt-G y3	L64-2584	L62-1027,cyt-G x (C(6) x T139)y3
Np *	L64-2709	L6 x (C(5) x Chief)

When the isoline has genes *Rps1* and *rxp* (from L2, Clark 63, L6, or L12) it is indicated by an asterisk(*) in the "Gene" column. ** Indicates probably *Rps1 rxp* but the testing for *rxp* is not completed.

C-2

<i>pc Pd1</i>	L65-44	(C(6) x PI 84.987) <i>pc</i> x (C(6) x PI 80.837) <i>Pd1</i>
<i>pc Ps</i>	L65-52	(C(6) x PI 84.987) <i>pc</i> x (C(6) x PI 91.160) <i>Ps</i>
<i>Pd1 Ps</i>	L65-90	(C(6) x PI 80.837) <i>Pd1</i> x (C(6) x PI 91.160) <i>Ps</i>
<i>lw1 t</i>	L65-600	C(6) x T176
<i>t</i>	-L65-601	"
<i>lbl</i>	L65-701	C(6) x PI 196.166
<i>f</i>	L65-763	C(6) x PI 83.945-4
<i>dt1 e2 Lf1</i>	L65-774	C(6) x PI 86.024
<i>dt1 e2</i>	L65-778(for L64-1477)	"
<i>dt1</i>	-L65-792	C(6) x PI 83.945-4
<i>r</i>	-L65-1068	C(6) x Higan
<i>w1</i>	-L65-1077	C(6) x PI 83.945-4
<i>fe</i>	L65-1255(L19)	C(6) x PI 54.619
<i>r *</i>	L65-1914	L6 x L11, <i>I r</i>
<i>E1 t</i>	L65-3366	C(6) x T175
<i>i *</i>	L66-14	mutation in Clark 63 received in 1965 from Portageville, MO
<i>i r</i>	L66-17	<i>i</i> -mutation in L11, <i>I r</i> found in 1965 at Urbana
<i>Rps1</i>	L66-180	PI 101.404A x C(6)
wild cytoplasm	L66-183	"
<i>td *</i>	L66-228	L6(6) x Sooty
<i>td</i>	L66-260	C(6) x PI 91.160
<i>E1 t e2</i>	L66-432	L62-1932, <i>e2</i> x L65-3366, <i>E1 t</i>
<i>dt1 E1 t e2</i>	L66-531(for 503)	L64-1437, <i>dt1 e2</i> x L65-3366, <i>E1 t</i>
<i>dt1 E1 t</i>	L66-546	"
<i>t</i>	L67-483	C(6) x Higan
<i>pal pa2 Ps-s</i>	L67-495	"
<i>pal pa2</i>	L67-497	"
<i>pal pa2 S</i>	L67-509	"
<i>S</i>	L67-592	"
wild cytoplasm	L67-1189	PI 101.404B x C(6)
<i>E1</i>	L67-1474	C(6) x T175
<i>lw1</i>	L67-1749	C(6) x T176
<i>P1 pc</i>	L67-3124	(C(6) x PI 84.987) <i>pc</i> x L62-1385, <i>P1</i>
<i>P1 Ps</i>	L67-3127	L62-1385, <i>P1</i> x (C(6) x PI 91.160) <i>Ps</i>
<i>dt1 s-t</i>	L67-3207	(C(6) x Chief) <i>s-t</i> x L63-3297, <i>dt1</i>
<i>Dt2 s-t</i>	L67-3224	(C(6) x Chief) <i>s-t</i> x L62-1251, <i>Dt2</i>
<i>Dt2 e2</i>	L67-3232	L62-1932, <i>e2</i> x L62-1251, <i>Dt2</i>
<i>e2 s-t</i>	L67-3243(for 3246)	L62-1932, <i>e2</i> x [L6 x (C(5) x Chief)] <i>s-t</i>
<i>i</i>	L67-3469	mutation in Clark found in 1954 at Urbana
<i>i *</i>	L67-3472	mutation in Clark 63 found in 1965 Illinois foundation seeds
<i>k1</i>	L67-3479	mutation in Clark from Ames, IA in 1956
<i>k1 *</i>	L67-3480	mutation in 1965 Illinois foundation seeds of Clark 63
<i>k2</i>	L67-3483(S57-3491)	mutation in x-rayed Clark at Columbia, MO
<i>i r *</i>	L67-3484	L66-14, <i>i *</i> x L12, <i>I r *</i>
<i>P1 Pd1</i>	L67-3770	L62-1385, <i>P1</i> x (C(6) x PI 80.837) <i>Pd1</i>
<i>i(wild)</i>	-L68-236	PI 101.404A x C(6)
<i>i Rps1</i>	-L68-237	"
<i>l2</i>	L68-1013	C(6) x Higan
<i>l2 t</i>	L68-1017	"
semi-wild cytoplasm		L68-1306PI 65.388 x C(6)
<i>L1 *</i>	L68-1562	L6(6) x Seneca
<i>w4 *</i>	L68-1774	L6(6) x (Laredo x Harosoy)
<i>-c Ps-s</i>	L68-1864	(C(6) x Higan) <i>Ps-s</i> x L63-2435, <i>pc</i>

C-3

I Pl r	L68-2045	L62-1058, I x (C(6) x T145)Pl r
I r t w1 *	L68-2056	L12 x L64-2281 sib, t w1
r w1 *	L68-2061	"
r t w1 *	L68-2063	"
i t	L68-2073	L67-3469, i x L64-2281 sib, t w1
i w1	L68-2077	"
kl t1	L68-2082	L67-3479, kl x L64-2281 sib, t w1
kl w1	L68-2085	"
kl t w1	L68-2093	"
I kl r	L68-2105	L67-3479, kl x L12, I r*
kl r	L68-2106	"
I kl	L68-2130	"
B1 i	L69-4544	L67-3469, i(6) x Sooty
i k *	L69-4607	L66-14, i* (6) x Black Eyebrow
G d1	L69-4659	C(6) x Columbia
d2	L69-4662	"
d1 d2	L69-4663(for L67-1000)	"
G d2	L69-4667(for 4666)	"
y9 *	L69-4755	L6(6) x T135
td w1 *	L69-4775	L6(6) x Seneca
w1 *	L69-4776	"
l2 w1	L69-4814	C(6) x T204
Im I r *	L69-5338, L69-5343	L12(6) x Hawkeye
Im r *	L69-5366	"
p2 I r *	L70-4049	L12(6) x T31
Rpm *	L70-4170	L12 x (Clark 63(5) x Kanrich)
r Rpm *	-L70-4186	"
I r Rpm *	L70-4190(for 4191)	"
i-k *	L70-4204(for 4209)	L66-14, i*(6) x Black Eyebrow
ln lo	L70-4313	(C(6) x T204)ln x L62-1615, lo
td *	L70-4404	L6(6) x Grant
L1 *	-L70-4413	L6(6) x (Laredo x Harosoy)
W3 w4 *	L70-4422	"
dt1 E1 t e2 *	L70-4478	L12 x L66-503, dt1 E1 t e2
i t w1	L70-4497	L67-3469, i x L64-2281 sib, t w1
I r t *	L70-4543	L12(6) x Hawkeye
pa2	L70-4558	C(6) x Higan
pa2 Ps-s	L70-4566	"
lo I r *	L70-4611	L12 x L62-1615, lo
ln *	L70-4629	L12 x L62-1579, ln
heterozygous for translocation	-L70-9283	PI 101.404B x C(6)
Ps *	L71-149	L12 x L63-2999, Ps
e2 e3	L71-920	L63-3117, e2 x L63-2404, e3
Dt2 S	L71-1284	L67-592, S x L62-1251, Dt2
Dt2 E1 t e2	L71-1363	L62-1251, Dt2 x L66-432, E1 t e2
e2 S	L71-1374	L67-592, S x L66-432, E1 t e2
E1 t e2 S	L71-1378	"
E1 t S	L71-1388	"
dt1 E1 t e2 s-t	L71-1403	L66-531, dt1 E1 t e2 x L67-3246, e2 s-t
dt1 ln	-L71U-659	L63-3297, dt1 x L62-1579, ln
e2 *	L72-1495	L6 x L63-3117, e2
e2 wm *	L72-1566	L6(6) x T235
e2 t *	L72-1568	"
e2 Im *	L72-1582	L6 x (L12(5) x Hawkeye)
E1 t *	L72-1630	L6 x L65-3366, E1 t

C-4

dt1 L1	L72-1727	(L6(6) x Seneca)L1 * x L63-3297,dt1
dt1 *	L72-1737	L6 x L63-3297
dt1 S	L72-1745	L67-592,S x L63-3016,dt1
E1 t e2 s-t	L72-1832	L66-531,dt1 E1 t e2 x L67-3246,e2 s-t
dt1 E1 t e2 Im *	L72-1853	(L12(6) x Hawkeye)Im * x L66-531,dt1 E1 t e2
E1 t e2 Pd1	L72-1879	L66-531,dt1 E1 t e2 x L62-1686,Pd1
dt1 E1 t e2 Pd1	L72-1885	"
E1 t s-t	L72-1893	L65-3366,E1 t x L64-1731,s-t Np
dt1 E1 t s-t	L72-1903	L66-531,dt1 E1 t e2 x L67-3207,dt1 s-t
Y11 y11	L72-1937(for L65-1237)	C(6) x T219H
n r *	L72-1977	L6(6) x Soysota
n *	L72-1987	"
i o r *	L72-2004	L67-3484,i r*(6) x Ogemaw
i r-m *	L72-2040	L67-3484,i r*(6) x PI 91.073
ln I r *	L72-2111	L12 x L62-1579,ln
I r rj1 *	L72-2133	L12 x L63-1889,rj1
Lf1 *	L72-2157	L12 x L64-1344,Lf1
wm *	L72-2181	L6(6) x T235
wm t *	L72-2210	"
d1 d2 p2 *	L72-2824	(L12(6) x T31)p2 * x (C(6) x Columbia)d1 d2
d1 d2 p2 I r *	L72-2832	"
ln Pd1	-L72U-4191	L62-1579,ln x L62-1686,Pd1
dt1 ln Rps1	-L72U-4282	L62-1579,ln x (L63-3297,dt1 x Clark 63)
dt1 Pd1	-L72U-4304	L63-3297,dt1 x L62-1686,Pd1
dt1 Pd1 *	-L72U-4317	Clark 63 x (L63-3297,dt1 x L62-1686,Pd1)
dt1 i r *	-L73-671	L67-3484,i r*(6) x PI 91.073
e2 I r *	-L73-681	L12(6) x Harosoy,e2
e2 I r *	-L73-695	L12(6) x Chippewa,e2
e2 I Im r *	L73-753	L12(6) x Hawkeye,e2 Im
e2 I r *	L73-760	"
Dt2 *	L73-811	L6 x L62-1251,Dt2
dt1 Dt2	L73-879	L62-1251,Dt2 x L63-3016,dt1
dt1 e2 s-t	L73-904	L66-531,dt1 E1 t e2 x L67-3246,e2 s-t
dt1 E1 t e2 I r *	L73-944	(L12(6) x Hawkeye)Im * x L66-531,dt1 E1 t e2
Dt2 E1 t	L73-980	L62-1251,Dt2 x L66-432,E1 t e2
F1 i *	L73-1004	L6 x (L67-3472,i*(5) x PI 47.131)
ab *	L73-1018	L6(6) x Kingwa
Pb *	L73-1034	"
Pd1 *	L73-1046	L12 x L62-1686,Pd1
r rj1 *	L73-1054	L12 x L63-1889,rj1
t td	L73-1071	L67-483,t x L66-228,td*
lf2 *	L73-1087	L6(6) x T255
pal pa2 pc	L73-1101	L67-497,pal pa2 x L63-2435,pc
pal pa2 Pd1	L73-1118	L67-497,pal pa2 x L62-1686,Pd1
pal pa2 Ps	L73-1144	L67-497,pal pa2 x L63-2999,Ps
dt1 ln Pd1	-L73U-2774	(L63-3297,dt1 x L62-1579,ln) x (L63-3297,dt1 x L62-1686,Pd1)
ms1 *	L74-03	L6(6) x T260H
s-t *	-L74-373	L12 x (C(6) x Chief)
e2 s-t *	-L74-434	L6 x L67-3246,e2 s-t
E1 t e3	-L74-441	L63-2404,e3 x L65-3366,E1 t
cyt G y9 **	-L74-824	L62-1027,cyt-G x L69-4755,y9*
cyt G Y11 y11	-L74-826	L62-1027,cyt-G x L65-1237,Y11 y11
d1 d2 y9 **	-L74-836	(C(6) x Columbia)G d1 d2 x L69-4755,y9*
G d1 d2 Y11 y11	-L74-838	(C(6) x Columbia)G d1 d2 x L65-1237,Y11 y11
G d1 d2 y3	-L74-851	L67-1000,d1 d2 x L63-2346,y3
d1 d2 y7 y8	-L74-852	L69-4663,d1 d2 x L63-1792,y7 y8

C-5

chromosome	-L74-1060(A72-1036)	PI 101.404B x C(6)
translocation		
i-k o r *	L74-1089	L69-4607,i-k* x (L67-3484,i r*(5) x Ogemaw,o)
ln Pd1 Rps1	-L74U-6710	L62-1579,ln x (Clark 63 x L62-1686,Pd1)
Rps1-c	-L75-3901	C(6) x Arksoy
i-k r t *	-L75-6426	L68-2063,r t w1* x L70-4209 sib,i-k*
i-k r w1 *	-L75-6433	"
i-k r t w1 *	-L75-6439	"
Rpm	-L75-6551	Clark x (Clark 63(6) x Kanrich)
e2 hb	-L75-6631	L72-1495,e2*(6) x PI 229.342
Pd2 *	-L75-6648	L6(6) x T264
ab *	-L75-6697	L6(6) x PI 47.131
dt1 E1 t e3	-L76-865	L63-2404,e3 x L66-546,dt1 E1 t
mi G t *	-L76-1010	L6 x [L68-2063,r t w1*(5) x (Laredo x Harosoy)]
		L68-2063,r t w1* x L70-4209,i-k*
i-k t *	-L76-1101	"
i-k w1 *	-L76-1113	"
i-k t w1 *	-L76-1122	"
v1 *	-L76-1149	L6(6) x T93
Rpm *	-L76-1169	L6 x (Clark 63(6) x Kanrich)
pal	-L76-1291	C(6) x Higan
Rps2 Rj2 rj2	-L76-2060	C(6) x L70-6494 (H(5) x D54-2437)
e2 12 Rps1-c rxp	-L76-2096	L6(2) x (L63-3117,e2(4) x PI 229.342)
12 Rps1-c rxp	-L76-2100	"
Rps1-c rxp	-L76-2107	L6(6) x PI 229.342
Rps1-k	-L77-2015	C(6) x Kingwa
dt1 Rps1-b hm	-L77-2050	C(6)x Harrel
Rps1-b	-L77-2061	"
1b1 Lb2	-L77-2654	L65-701,1b1(6) x PI 196.166
Rsv1 *	-L78-434	L6(6) x PI 96.983
hb *	-L78-3263	L6(6) x PI 229.342
ms2 *	-L79-1308	L6(6) x T259H
Rps1-c	-L79-1380	C(6) x Higan
12 Rps1-c	-L79-1404	C(6) x Arksoy
lw1 t Lw2	-L79-1685	L65-600,lw1 t(6) x T117,Lw2
Dt2 S *	L79-1800(for L74-387)	L6 x (L67-592,S x L62-1251,Dt2)
Pd1 Pd2 rxp?	-L79-1815	L62-1686,Pd1 x (L6(6) x T264)Pd2
dt1 E1 t e2 e3	-L80-5879	L70-4478,dt1 E1 t e2* x L71-920,e2 e3
dt1 e2 e3 rxp?	-L80-5882	"
E1 t e2 e3	-L80-5914	"
pal pa2	-L81-4651	L70-4558,pa2 x L76-1291,pa1
e2 *	-L81-4659	L6(6) x Calland
rj1 *	-L81-4858	L6(2) x L63-1889
ti *	-L81-4871	L6(6) x PI 157.440
w4 *	-L81-4945	L6(6) x PI 81.763
I r w1 Rps1 rps1	-L81-5080	L12 x L64-2281 sib,t w1
r w1 *	L81-5082	"
i r t w1 *	-L82-2669	L66-14,i* x (L12 x L64-2281 sib,t w1)
i ti *	-L83-693	i mutation in L6(6) x PI 157.440
I t w1 **	-L83-841	L12 x L64-2281 sib,t w1
I t *	-L83-879	"
r t **	-L83-900	"
i r t *	-L83-930	L66-14,i* x (L12 x L64-2281 sib,t w1)
i r w1 *	-L83-942	"
dt1 L1	-L83-964	L68-1562,L1 x L63-3016,dt1
t w4 *	-L84-1135	L6(6) x (Laredo x Harosoy)

I wl **
 t
 I t *
 I t
 r t
 I t wl **
 I t wl
 k3

-L84-1231
 -L85-1467
 -L86-1078
 -L86-1084
 -L86-1092
 -L86-1096
 -L86-1098
 T238

L12 x L64-2281 sib,t wl
 C(6) x PI 84.987
 L12 x L64-2281 sib,t wl
 "
 "
 "
 "

mutation in x-rayed Clark found
 in 1956 at Columbia, Missouri

H-1

APPENDIX 2: HAROSOY ISOLINES ARRANGED BY LINE DESIGNATION

Harosoy = Ab cyt-Y D1 D2 Dt1 dt2 e1 e2 E3 e5 F Fe g Hm I K2 l1 L2 lf1 Ln Lo Lw1 lw2
 Mn Msl N np p1 P2 Pal Pa2 pb Pc pd1 ps r Rj1 rj2 rmd rps1 rps2 rps5 Rxp s t
 W1 w3 W4 Wm Y3 Y7 Y8 Y9 Y20

-	L58-266 (Harosoy)	Mandarin (Ottawa)(2) x AK (Harrow)
Rps1	L59-731(Harosoy 63)	H(8) x Blackhawk
rxp	L61-4094(L3)	H(6) x S54-1207
Rps1 rxp	L61-5047(L2)	Harosoy 63 x L3,rxp
cyt-G	L62-17	Medium Green x H(7)
Dt2	L62-364	H(6) x T117
l2	L62-542	H(6) x T145
l2 P1	L62-558	"
P1	L62-561	"
e3	L62-667(for L73-1543)	H(6) x T204
Pd1	L62-801	H(6) x PI 80.837
Dt2 E5	-L62-812	"
Ps	L62-880	H(6) x PI 91.160
Rps5 w1	-L62-904	"
w1	L62-906	"
Lf1	L62-956	H(6) x PI 86.024
dt1	L62-973	"
y3	L63-1016	H(6) x T139
R	-L63-1069	"
pc	L63-1097	H(6) x PI 84.987
ln	L63-1212	H(6) x T204
Dt2	L63-1397	H(6) x PI 80.837
wm Rps1	L63-1612	Harosoy 63 x [(T235(2) x (H(5) x Blackhawk)]
Lf1 ln	L64-1069	(H(6) x PI 86.024)Lf1 x (H(6) x T204)ln
w1	-L64-2139	H(6) x Peking
G d1 d2	L64-2489	H(6) x Columbia
E1	-L64-4103	"
E2	-L64-4584	H(6) x T117
E5	-L64-4830	H(6) x PI 80.837
pc Pd1	L65-25	(H(6) x PI 84.987)pc x (H(6) x PI 80.837)Pd1
pc Ps	L65-34	(H(6) x PI 84.987)pc x (H(6) x PI 91.160)Ps
Pd1 Ps	L65-60	(H(6) x PI 91.160)Ps x (H(6) x PI 80.837)Pd1
pal Ps-s	L65-237	H(6) x Higan
lo	L65-372	H(6) x T204
lw1	L65-461	H(6) x T176
R	L65-540	"
i-i	-L65-556	"
f	L65-756	H(6) x PI 83.945-4
i-i	-L65-1053	H(6) x T117
Dt2 i-i	L65-1058	"
rj1	L65-1274(L14)	H(6) x T201
Np	L66-704(L17)	H(6) x (C(6) x Chief)
T	L66-707	H(6) x Clark
Np T	L66-713	H(6) x L9(Clark-Np)
i-i Np	L66-721	"
fe	L66-731(L18)	H(6) x PI 54.619

rj1 * L66-2470
i-i L67-38
dt1 L67-153
Ps-s L67-166
l2 S L67-225
l2 L67-226
S L67-234
pal L67-271
i-i -L67-949
G d2 L67-971
*Dt2 ** L67-1250
i-i R lwl L67-1687
i-i R L67-1695
E1 T L67-2324
P1 pc L67-3099
P1 Pd1 L67-3101
P1 Ps L67-3104
dt1 Dt2 L67-3256
Dt2 Lf1 ln L67-3298

i L67-3388
*i ** L67-3396
y7 y8 L68-560
*L1 ** L68-582
E1 L68-694
Rps1 rxp L68-758
G d1 L69-4265(for 4268)
d2 L69-4266
d1 d2 L69-4267
*y9 ** L69-4318
*L1 ** -L69-4428
pal pa2 L69-6095
*p2 ** L70-4001
*d1 d2 p2 ** L70-4037
pa2 L70-4112(for 4119)
ln lo L70-4136
*Pd1 ** L71-46
E1 T e3 L71-802
E1 T S L71-1106
dt1 E1 T L71-1116
E1 T E2 -L71L-3004
E1 T E5 -L71L-3015
*W3 w4 ** L72-1078
*w4 ** L72-1138
*n ** L72-1140
*dt1 ** L72-1177
*S ** L72-1198
dt1 S L72-1228
Dt2 S L72-1241
*E1 T ** L72-1304
ln Pd1 -L72D-4028
ln Pd1 Rps1 -L72D-4029
ln Rps1 -L72D-4045

H-2

L2 x (H(6) x T201)
 H(6) x Clark
 H(6) x Higan
 "
 "
 "
 "
 "
 H(6) x Columbia
 "
 L2 x (H(6) x T117)
 H(6) x T176
 "
 H(6) x PI 196.166
 (H(6) x PI 84.987)*pc* x L62-561,*P1*
 (H(6) x PI 80.837)*Pd1* x L62-561,*P1*
 (H(6) x PI 91.160)*Ps* x L62-561,*P1*
 (H(6) x T117)*Dt2* x L62-973,*dt1*
 [(H(6) x T117)*Dt2* x (H(6) x T204)*ln*] x
 [(H(6) x T117)*Dt2* x (H(6) x PI 86.024)*Lf1*]
 mutation in Harosoy found in 1959 at Urbana
 mutation in L2 found in 1963 at Shabbona, IL
 H(6) x T138
 L2(6) x Seneca
 H(6) x PI 196.166
 H(4) x L2,*Rps1 rxp*
 H(6) x Columbia
 "
 "
 L2(6) x T135
 L2(6) x PI 81.763
 H(6) x Higan
 L2(6) x T31
 (L2(5) x T31)*p2** x (H(6) x Columbia)*d1 d2*
 H(6) x Higan
 (H(6) x T204)*ln* x (H(6) x T204)*lo*
 L2 x L62-801,*Pd1*
 L62-667,*e3* x L67-2324,*E1 T*
 L67-234,*S* x L67-2324, *E1 T*
 L67-2324,*E1 T* x L67-153,*dt1*
 L67-2324,*E1 T* x L64-4584,*E2*
 L67-2324,*E1 T* x L64-4830,*E5*
 L2(6) x Laredo
 "
 L2(6) x Soysota
 L2 x L62-535,*dt1* (H(6) x T145)
 L2 x L67-234,*S*
 L67-234,*S* x L67-153,*dt1*
 L67-234,*S* x L63-1397,*Dt2*
 L2 x L67-2324,*E1 T*
 L63-1212,*ln* x L62-801,*Pd1*
 (L63-1212,*ln* x Harosoy 63) x L62-801,*Pd1*
 L63-1212,*ln* x Harosoy 63

dt1 ln -L72D-4110
dt1 ln Pd1 -L72D-4169

d1 L73-54
*ab ** L73-67
*Pb ** L73-79
*Dt2 S ** L73-105
dt1 Pd1 -L73D-2733
dt1 ln Rps1 -L73D-2743

*E2 T ** -L74-21
*E2 ** -L74-27
Dt2 E1 T L74-59
E2 E5 -L74-66
Dt2 E1 T S -L79-842
pal pa2 -L81-4075
Rps2 Rmd Rj2 -L82-1449
E2 e3 -L84-307
e3 E5 -L84-337
Rps1-c hm? -L85-129
12 Rps1-c hm? -L85-144
Rps1 Rps2 Rmd Rj2 -L86-493
E2 E5 -L86L-4
wm T235
k2 T239
mm T251H

y20 k2 T253
ms1 (Tonica) T267H

H-3

L63-1212, *ln* x L62-535, *dt1* (H(6) x T145)
 (L62-535, *dt1* x L62-801, *Pd1*)
 x (L62-535, *dt1* x L63-1212, *ln*)
 L69-4268, *G d1* x L69-4267, *d1 d2*
 L2(6) x Kingwa
 "
 (L2 x L67-234) *S** x L67-1250, *Dt2**
 L62-535, *dt1* x L62-801, *Pd1*
 (L63-1212, *ln* x Harosoy 63) x
 (L62-535, *dt1* x Harosoy 63)
 L2(6) x Clark
 "
 L67-2324, *E1 T* x L67-1397, *Dt2*
 L64-4584, *E2* x L64-4830, *E5*
 L71-1106, *E1 T S* x L74-59, *Dt2 E1 T*
 L67-271, *pal* x L70-4112, *pa2*
 H(6) x D54-2437
 L64-4584, *E2* x L73-1543, *e3*
 L64-4830, *E5* x L73-1543, *e3*
 H(6) x Higan
 "
 L2, *Rps1* x (H(5) x D54-2437) *Rps2 Rmd Rj2*
 L64-4830, *E5* x L74-27, *E2**
 mutation in Harosoy found in 1957 at Urbana
 mutation in Harosoy found in 1961 at Urbana
 mutation in Harosoy(5) x T139 found in 1961
 at Urbana
 mutation in T239(*k2*) found in 1963 at Urbana
 mutation in Harosoy found in 1955 at
 Tonica, Illinois

W-1

APPENDIX 3: WILLIAMS ISOLINES ARRANGED BY LINE DESIGNATION

Williams (Wm) - Dt1 dt2 E2 Ep Hm i-i im l2 Lx1 Ms2 rj2 Rmd rpm rppl rpp2 rpp4 rps1
rps2 rps3 rps4 rps5 rpv rsv1 rsv2 Sun T Ti-a w1

ms2	L74-01	Wm(6) x T259H
Dt2	L74-189(Will)	Wm(6) x Clark(6) x T117)
Dt2 ep	-L74-221(Will)	"
Rps1-c	L75-3735(Williams 79)	Wm(6) x Lee 68
e2	-L75-6111	Wm(6) x [SL12,Wayne-I r Rpm Rps1(6) x (Clark(6) x PI 86.024)e2]
Rpm Rps1	-L75-6141(for L75-6125)	Wm(6) x SL12,Wayne-Rpm Rps1; - Wm x Union
Rps2 Rj2	-L76-1988(for L76-2013)	Wm(6) x (Harosoy(5) x D54-2437)
I Rps2 Rj2	-L76-1994	"
t Rps2 Rj2	-L76-2023	"
Rps1-c	-L77-1727	Wm(6) x (Clark 63(3) x PI 229.342)
Rps1-k	L77-1794(Williams 82)	Wm(7) x Kingwa
Rps1-b hm	-L77-1863 (for 1818)(L26)	Wm(7) x Harrel
Im	-L77-5632(for L75-6867)	Wm(6) x L69-5343,Clark -I r Im*
Rsv1	-L78-379(L25)	Wm(6) x PI 96.983
L2 Im	-L78-3079	Wm(6) x L69-5343,Clark I r Im*
L2	-L78-3083	"
cytoplasm	-L78-3130	Wisconsin Black x Wm(6)
W1 Rps1-c	-L79-908	Wm(6) x Lee 68
Dt2 Rps1-k	-L79-1250(for L79-1224)	Will x Wm 82
Dt2 Im	-L79-1270	Will x L75-6867,Im
Dt2 Rsv1	-L80-5227(for L80-5230)	Will x (Wm(6) x PI 96.983)
W1 Rps1-c	-L80-5372	Wm(6) x (Clark 63(3) x PI 229.342)
Rpm	-L80-5427	Wm x L75-6125,Rpm Rps1; - Wm(2) x Union
Im Rps1-c	-L80-5452	Wm 79,Rps1-c x L75-6867,Im
Dt2 Rpm Rps1	-L81-4308(for L79-1178)	Will x L75-6125,Rpm Rps1
Rps1-c Rps2 Rj2	-L81-4352	Wm 79,Rps1-c x L76-2013,Rps2 Rj2
Rps1-k Rsv1	-L81-4420	L78-379,Rsv1 x Wm 82
ti Rps1-k	-L81-4590(= Kunitz)	Wm 82(6) x PI 157.440
Dt2 Rpm Rsv1	-L82-753	L79-1178,Dt2 Rpm Rps1 x L80-5227,Dt2 Rsv1
Dt2 Rpm Rps1-k	-L82-1560	L79-1178,Dt2 Rpm Rps1 x L79-1224,Dt2 Rps1-k
Rpm Rps1-c Rps2 Rj2	-L82-1858	L81-4352,Rps1-c Rps2 Rj2 x (L75-6141,Rpm Rps1 x L76-1988,Rps2 Rj2)
Ti-b	-L82-2020	Wm(6) x Jefferson
rmd Ti-b	-L82-2024	"
dt1 Ti-c	-L82-2045	Wm(6) x PI 196.172
Ti-c	-L82-2051	"
Rps3	-L83-570	Wm(6) x PI 86.972-1
Dt2 Rpm Rps1 Rsv1	-L83-4483	L79-1178,Dt2 Rpm Rps1 x L80-5227,Dt2 Rsv1
Dt2 Rps1 Rsv1	-L83-4494	"
Dt2 Rps1-k Rsv1	-L83-4527	L79-1224,Dt2 Rps1-k x L80-5227,Dt2 Rsv1
Rpm Rsv1	-L83-4744	L78-379,Rsv1 x L80-5493,Rpm Rps1-k
Rpm Rps1-k Rsv1	-L83-4752	"
i (not Ti-b)	-L84-2003	mutation in Wm(6) x Jefferson found in 1982 at Urbana
rmd	-L84-2237	Wm(6) x (Clark 63(2) x PI 86.150)
dt1	-L85-2029	"
sun	-L85-2196	Wm(6) x PI 229.324
Rpv	-L85-2308	Wm(6) x Dorman
Rps4	-L85-2352	Wm(6) x PI 86.050

W-2

<i>Rpp1 Rps1-k</i>	-L85-2378	Wm 82(6) x PI 200.492
<i>Rps5</i>	-L85-3059	Wm(6) x PI 91.160
<i>lx1</i>	-L86-1436	Wm(6) x PI 408.251
<i>Rpp2 Rps1-k</i>	-L86-1752	Wm 82(6) x PI 230.970
<i>Rpp4 Rps1-k</i>	-L87-0482	Wm 82(6) x PI 459.025
<i>i</i>	-L88-5492	mutant found in Williams in 1980 from a farm on Windsor Road, Champaign, IL
<i>i Rps1-c</i>	-L88-5495	mutant found in Williams(6) x Lee 68 in 1975 at Urbana (L75-3774)
<i>dt1 i Ti-c</i>	-L88-8012	i-mutant selection from Wm(6) x PI 196.172
<i>dt1</i>	-L88-8025	Wm(6) x PI 408.251
<i>rmd</i>	-L88-8226	Wm(6) x PI 86.972-1
<i>Rsv2</i>	-L88-8431	Wm(6) x Raiden, PI 360.844
<i>r Rsv2</i>	-L88-8440	"
<i>Rpm Rps1-k</i>	-L88-8488 (for L80-5493)	Wm 82, <i>Rps1-k</i> x (Wm x L75-6125, <i>Rpm Rps1</i>)
<i>Rpm Rps1-b hm</i>	-L88-8502	L75-6125, <i>Rpm Rps1</i> x L77-1818, <i>Rps1-b hm</i>
<i>Dt2 Rpm Rps1-k Rsv1</i>	-L88-8739	L79-1224, <i>Dt2 Rps1-k</i> x (L79-1178, <i>Dt2 Rpm Rps1</i> x L80-5230, <i>Dt2 Rsv1</i>)

APPENDIX 4. DESCRIPTION OF GENES TRANSFERRED

<i>ab</i>	delayed leaf abscission
<i>B1</i>	bloom on seed coat (Clark and Harosoy are apparently <i>b1 B2 B3</i>)
<i>cyt-G</i>	cytoplasmically inherited "stay-green" in plant and seeds
<i>d1 d2</i>	genetically inherited "stay-green" in plant and seeds
<i>dt1</i>	determinate stem
<i>Dt2</i>	semi-determinate stem
<i>E1</i>	late maturity
<i>e2</i>	early maturity (Harosoy is <i>e2</i>)
<i>e3</i>	early maturity and low photoperiod sensitivity to incandescent light
<i>E5</i>	late maturity
<i>ep</i>	low peroxidase activity in seeds
<i>f</i>	fasciated stem
<i>fe</i>	iron inefficient
<i>F1</i>	brown flecks on black seeds
<i>G</i>	green seed coat
<i>hb</i>	bentazon sensitive
<i>hm</i>	metribuzin sensitive
<i>i</i>	black seed coat with <i>R T</i> , brown with <i>r T</i> , imperfect black with <i>R t W1</i> , or buff with <i>Rt w1</i> or <i>r t</i>
<i>i-i</i>	above color in hilum only (Clark and Williams are <i>i-i</i>)
<i>i-k</i>	above color in saddle pattern
<i>I</i>	light hilum (black and imperfect black are diluted to gray; brown and buff are diluted to clear) (Harosoy is <i>I</i>)
<i>Im</i>	non-mottling (in recent years at Urbana lines with this genotype have mottled)
<i>k1</i>	black (brown with <i>r</i>) saddle on seeds
<i>k2</i>	tan saddle on seeds
<i>k3</i>	black (brown with <i>r</i>) saddle on seeds
<i>L1</i>	black pod
<i>l2</i>	tan pod (Williams is <i>l2</i>)
<i>lb1 lb2</i>	bullate leaf, no effect individually (Clark is <i>Lb1 lb2</i>)
<i>Lf1</i>	five leaflets
<i>lf2</i>	seven leaflets
<i>ln</i>	narrow leaflet, four-seeded pod
<i>lo</i>	oval leaflet, few-seeded pod
<i>lw1 lw2</i>	wavy leaf (no effect with <i>T</i> or individually), Clark & Harosoy are <i>Lw1 lw2</i>
<i>lx1</i>	no lipoxygenase-1 in seeds
<i>mi</i>	minute hilum with <i>t</i>
<i>mn</i>	miniature plant
<i>ms1</i>	male sterile, somewhat female sterile
<i>ms2</i>	male sterile
<i>n</i>	abnormal hilum abscission
<i>Np</i>	phosphorus tolerant
<i>σ</i>	reddish brown seed pigment with <i>r T</i>
<i>P1</i>	glabrous
<i>p2</i>	puberulent, dwarf, seed coat cracks, partly male sterile
<i>pal pa2</i>	appressed pubescence (upper leaf surface)
<i>pal</i>	semi-appressed pubescence (upper leaf surface)
<i>pa2</i>	no effect without <i>pal</i>
<i>Pb</i>	sharp pubescence tip
<i>pc</i>	curly pubescence
<i>Pd1</i>	dense pubescence
<i>Pd2</i>	dense pubescence
<i>Ps</i>	sparse pubescence
<i>Ps-s</i>	semi-sparse pubescence

<i>R</i>	black seed pigment with <i>T</i> , imperfect black with <i>t W1</i> , or buff with <i>t w1</i> (Clark and Williams are <i>R</i>)
<i>r</i>	brown seed pigment with <i>T</i> or buff with <i>t</i> (Harosoy is <i>r</i>)
<i>r-m</i>	black and brown striped seed coat with <i>T</i> or imperfect black and buff striped with <i>t</i>
<i>rj1</i>	non-nodulating
<i>Rj2</i>	ineffective nodulation with Rhizobium strains b7, b14, or b122
<i>rmd</i>	susceptible to powdery mildew (Harosoy is <i>rmd</i>)
<i>Rpm</i>	resistant to downy mildew
<i>Rps1 to 5</i>	resistant to phytophthora rot
<i>Rpl1,2,4</i>	resistant to soybean rust
<i>Rpv</i>	resistant to peanut mottle virus
<i>Rsv1, 2</i>	resistant to soybean mosaic virus
<i>rxp</i>	resistant to bacterial pustule (Wayne and Williams are <i>rxp</i>)
<i>S</i>	short internode
<i>s-t</i>	long internode
<i>sun</i>	no urease in seeds
<i>t</i>	gray pubescence (Harosoy is <i>t</i>)
<i>td</i>	light tawny to near-gray pubescence with <i>T</i>
<i>ti</i>	no Kunitz trypsin inhibitor in seeds
<i>Ti-b,c</i>	Kunitz trypsin inhibitor variants in seeds
<i>v1</i>	variegated leaf
<i>w1</i>	white flower (Williams is <i>w1</i>)
<i>W3 w4</i>	purple throat flower color
<i>w4</i>	near-white flower
<i>wm</i>	magenta flower
<i>y3</i>	leaves turning rusty yellow with <i>g</i>
<i>y7 y8</i>	new leaves very light green in cool weather, no effect individually
<i>y9</i>	yellow-green plant
<i>Y11 y11</i>	yellow-green plant, <i>y11 y11</i> is a lethal, orangish yellow seedling
<i>y20</i>	yellow-green plant

Credits:

H. R. Boerma, University of Georgia, conducted progeny tests for the transfer of *Rpv* (peanut mottle resistance) to Williams giving isolate L85-2308.

R. I. Buzzell, Agriculture Canada, Harrow, identified the gene for earliness in L62-667 (Harosoy) and L63-2404 (Clark) as *e3*, and the gene in L62-904 as *Rps5* for phytophthora resistance.

R. L. Cooper, USDA and University of Illinois (now Ohio Agricultural Research and Development Center) developed the 2- and 3- gene combinations in Clark and Harosoy involving genes *dt1* (determinate stem), *ln* (narrow leaflet), and *Pd* (dense pubescence) (all lines with L__D- and L__U- prefixes).

T. E. Devine, USDA, Beltsville ARC, identified the *Rps2*-linked gene *Rj2* (controlling a Rhizobium response) in L82-1449 (Harosoy-*Rps2*), L80-5015 and L82-1187 (Corsoy-*Rps1-k Rps2*), and L76-1988 (Williams-*Rps2*) and showed that L76-2060 (Clark-*Rps2*) contained a crossover which had the original Clark allele *rj2*. He developed the *Rj2* and *Rj4* Clark isolines BARC-4 and -2.

R. W. Howell, USDA and University of Illinois, conducted progeny tests for the transfer of genes *fe* (iron inefficient) and *Np* (phosphorus tolerance) to Clark and Harosoy.

T. Hymowitz, University of Illinois conducted the progeny tests for seed composition for the transfer of *ti* (Kunitz trypsin inhibitor null) to Clark 63 (L6), Amsoy 71, and Williams 82, and *ep*, *lxl*, *sun*, *Ti-b*, and *Ti-c* to Williams.

B. A. McBlain, University of Illinois and now Ohio Agricultural Research and Development Center, identified the gene in L64-4830 (Harosoy BC) as *E5*.

J. S. Melching, USDA, Frederick, MD, conducted the progeny tests for rust resistance (*Rpp1*, *Rpp2*, and *Rpp4*) transfers to Williams 82.

R. G. Palmer, USDA and Iowa State University, identified the cause of semisterility in Clark isolate L70-9283 as a chromosome translocation.

L. M. Wax, USDA and University of Illinois, conducted progeny tests for bentazon sensitivity *hb* in Clark isolate L78-3263 and for metribuzin sensitivity *hm* (linked to *Rps1*) in L85-129 (Harosoy-*Rps1-c*) and L77-1863 (Williams-*Rps1-b*) and demonstrated that L77-2061 (Clark-*Rps1-b*) was a crossover and had the original allele *Hm*.

L. F. Williams (dec.), USDA and University of Missouri, conducted the backcrossing for downy mildew resistance for the Clark-*Rpm*, Chippewa-*Rpm*, and Wayne-*Rpm* isolines and for pustule resistance in L60-246 (Clark 63) and found the mutants *k2* and *k3* in Clark.

USDA NORTHERN SOYBEAN GERMPLASM COLLECTION REPORT

February 1991

The USDA Southern and Northern Soybean Germplasm collections were consolidated in February 1991. A single collection will now be maintained at Urbana, Illinois with R. L. Nelson as curator and C. J. Coble as assistant curator. Accessions in maturity groups V to X will still be grown at Stoneville, Mississippi and other locations at lower latitudes with the assistance of T. C. Kilen and E. E. Hartwig. All requests for seed and information on the collection should now be sent to the USDA Soybean Germplasm Collection, Department of Agronomy, University of Illinois, W-321 Turner Hall, 1102 South Goodwin Avenue, Urbana, Illinois, 61801, USA.

In 1990, a total of 5,063 seedlots were distributed from the USDA Soybean Germplasm Collection at the Urbana, Illinois location, in response to 313 requests from 35 states and 27 foreign countries. Seed orders were placed by 260 U.S. requestors for 3,817 seedlots and by 53 foreign requestors for the remaining 1,246. Numerous publications were sent in response to 40 requests for information about the collection. Additionally, 385 accessions were sent to the National Seed Storage Laboratory at Ft. Collins, Colorado as back-up samples of the collection.

Of the approximately 8700 *Glycine max* strains in the Urbana collection, 1613 were grown in 4-row plots in 1990 for germplasm maintenance. After being pure-lined in 1990, 76 new lines were added to the collection and are now available for distribution. Fifty-one of the additions were from China, 16 from Japan, 3 from Taiwan and 2 from the Republic of Korea.

The third and final year of general evaluation was completed for 513 accessions at Urbana and 43 accessions at St. Paul, Minnesota, with the assistance of J.H. Orf. Evaluation data will be summarized and published in a USDA Technical Bulletin in 1991 for PI 490.765 through PI 507.573.

Thirty germplasm maintenance plots of *Glycine soja* were grown in 1990. The 8 new lines added to the wild soybean collection were all from the Jilin Academy of Agricultural Sciences, Jilin, Peoples Republic of China. The current inventory of the USDA Wild Soybean Germplasm Collection consists of 900 accessions.

Glycine soja accessions were grown in a screen cage approximately 40 x 60 x 7 feet in 1990. The screen material was translucent with a 500 micron mesh. This screen causes an approximately 30% reduction in light transmission but excludes insects as small as aphids. This eliminates two serious pests of *G. soja* at Urbana, aphid-transmitted soybean mosaic virus and potato leaf hopper (*Empoasca fabae*). Plant height and seed yield of *G. soja* plants inside the cage were several fold greater than plants of the same genotype grown just outside the cage. The removal of these stresses also allows better expression of morphological differences among accessions and will assist in making more accurate descriptions of this material. A second, similar cage will be constructed and used in 1991.

Prior to planting in 1990, 98 accessions were received at Urbana. Of these, 15 *G. max* and 17 *G. soja* lines were sent from the Institute of Crop Germplasm Resources, Beijing, PRC; 21 soybeans collected in Nepal by an IBPGR-sponsored collection trip, via the Plant Introduction Office; 6 from N.I. Vavilov All-Union Institute of Plant Industry, Leningrad, USSR, via PIO; 30 from Japan, via John Konovsky of Washington State University, Pullman, WA; and 9 from PRC, via L.M. Mansur, Iowa State University, Ames, IA.

New *G. max* accessions received to date and to be planted in 1991 include 14 from the Department of Genetics, Voronezh State University, Voronezh, USSR; 60 from the Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Science, Beijing, PRC; 3 from Heilongjiang Academy of Agricultural Sciences, Heilongjiang, PRC and 2 from the National Institute of Agrobiological Resources, Ibaraki, Japan.

The current inventory of the USDA Perennial Glycine Germplasm Collection consists of 837 accessions representing 15 species. During the year, 14 accessions, all of which were collected in Australia, were received from CSIRO in Australia. Several accessions have proved difficult to multiply in this latitude. These have been sent, with the assistance of Dr. Henry Shands, to the USDA Tropical Agriculture Research Station, Mayaguez, Puerto Rico. The plants are cared for by Dr. Antonio Sotomayor. Of the total collection, 408 accessions are currently available for distribution. During 1990, 306 seedlots were sent in response to 14 requests from 3 states and 9 foreign countries. An additional 105 accessions were sent to the National Seed Storage Laboratory, Ft. Collins, Colorado, for back-up.

The USDA Southern and Northern Soybean Germplasm collections were consolidated in February 1991 and will be maintained as a single collection at Urbana, Illinois. Accessions in maturity groups V to X will still be grown at Stoneville, Mississippi and other locations at lower latitudes. All requests for seed should now be sent to the USDA Soybean Germplasm Collection, University of Illinois, Department of Agronomy, W-321 Turner Hall, 1102 South Goodwin Avenue, Urbana, Illinois, 61801, USA. With the consolidation of the collections, the number of seed requests that will be filled and the amount of information that will be managed will increase significantly at Urbana. Orders are now being processed using a local database management system which has been integrated with the GRIN. Soybean germplasm distribution data can be kept current in GRIN and is readily accessible locally. Using this system has reduced our costs for connect-time to GRIN. Currently, we are refining this system so that all site personnel can process orders quickly, easily and accurately. We are also looking into the feasibility of streamlining the inventory maintenance system and linking it to germplasm distribution records.

The type of storage container for the individual accessions has been changed. Because of the size and shape of the new container 10% more storage area is required but each container will hold more than twice as many seeds. This change will reduce the number of accessions that will need to be increased before the seed is ten years old.

Preparations have begun to have PI numbers assigned to all of the accessions in the collection, including cultivars, types and isolines. This will eliminate some of the confusion which arises when multiple inventory identifiers exist in GRIN. Toward this goal, GRIN accession records of the isolines now contain pedigree information and genetic codes. Genetic information has also been added to the accession records of the Genetic Type Collection.

C.J. Coble and R.L. Nelson
USDA-Agricultural Research Service
University of Illinois
1102 S. Goodwin Avenue
Urbana, Illinois 61801

SOYBEAN GERMPLASM CROP ADVISORY COMMITTEE REPORT

The Soybean Germplasm Crop Advisory Committee (CAC) held its annual meeting Feb. 18, 1991 at the Soybean Breeders Workshop in Memphis TN. Twelve of the 14 members were in attendance. Those elected to three-year terms were: Tom Devine, USDA ARS, Beltsville MD representing Molecular Biology and Cytogenetics; Bill Kenworthy, University of Maryland, College Park MD, representing Public Breeding from the North; and Lavone Lambert, USDA ARS, Stoneville MS, representing Entomology for a second consecutive three-year term. Dr. Devine was at the meeting. Also in attendance were Harvey Voldeng, Public Breeder, invited observer from Canada; Allan K. Stoner, Germplasm Services Laboratory; James Specht, University of Nebraska; Tommy Carter, USDA ARS, North Carolina State University; John Dayde, of France; Yantong Wang, Chinese Institute of Crop Germplasm Resources; and Hu Yunzhu, Nanjing Agricultural University, People's Republic of China.

Jim Orf, chairman, called the meeting to order and instructed the three Soybean CAC Subcommittees of Acquisition, Evaluation, and Operations, to meet and discuss pertinent issues and bring back subcommittee reports and recommendations to the entire committee. Subcommittee assignments were as follows:

Acquisition: H.R. Boerma, R.L. Nelson, J.G. Shannon; Evaluation: T.S. Abney, C.J. Coble, D.B. Egli, T.C. Kilen, L. Lambert, L.D. Young; Operations: C.J. Coble, E.E. Hartwig, J.H. Orf, R.G. Palmer, and J. Thorne.

After subcommittees' discussions, the meeting reconvened. Jim Orf reported that he had copies of minutes, reports, and other publications relative to germplasm including the report Managing Global Genetic Resources from the National Research Council for review by interested persons. Updates on both the southern and northern portions of the germplasm collection were given by Edgar Hartwig, Claudia Coble, and Randall Nelson. They reported that over 9,000 and 5,000 seed requests were filled from southern and northern germplasm collections, respectively, for US and foreign requestors. As of February, 1991, the USDA Northern and Southern Soybean Germplasm Collections have been combined and will be maintained as a single collection at Urbana IL. All seed requests should be made to Urbana. These changes will be published in Agronomy News, Diversity, and the Soybean Genetics Newsletter, to inform

germplasm users. Pure lining, evaluation, and seed increase for accessions in maturity groups V through X will continue to be coordinated at Stoneville MS. Most of this work will be handled at Stoneville with group IX and X being grown at Gainesville FL and Mayaguez PR, respectively. Dr. Hartwig expressed concern about soybean acquisitions collected from Nepal in 1984 concerning seed mixtures and the long lapse from the time it was collected until it was grown (1990). He reported that germplasm evaluation, by Dr. Lambert, of groups V through X of reaction to soybean looper, and reaction to races 3, 5, and 14 of soybean cyst nematode, by Dr. Lawrence Young, is nearly complete, with results in the process of being put on GRIN. Claudia Coble and Randall Nelson discussed the acquisition and evaluation of Northern germplasm. Acquisitions have been obtained from China, Nepal, USSR, Japan, Australia, and Indonesia. They reported that Glycine soja increases and morphological differentiation among accessions have been improved by growing them in cages. Cages exclude insects that transmit viruses and other diseases. Another cage is planned for use in 1991. Claudia reported that the three older publications listing information for groups 000-IV will be consolidated into one new report. Also, evaluation data for PI 490,765 through PI 507,573 will be published in a USDA Technical Bulletin in 1991. She also reported that PI numbers will be assigned to all of the accessions in the collection including cultivars, types, and isolines. This will eliminate confusion when multiple inventory identifiers exist in GRIN. Reports on the current collections are presented on pages 14-57 of this volume.

Allan Stoner presented an update of the GRIN (Germplasm Resources Information Network) System. He reported that the system continues to improve with more and more descriptive information being put in and inaccurate information deleted. There are more than 600 public users with access codes. Seed orders can now be placed electronically. A new computer and operating system will replace the current 10-year-old system, which is outdated. New data in this new system will also involve information on isozymes, RFLP's, and gene mapping. It will allow increased capacity and speed and will be easier to use with the ability to link with other germplasm systems around the world.

Subcommittee reports were given:

- 1) Acquisition Subcommittee: Randy Nelson reported from the acquisition subcommittee. The subcommittee suggested that when segregating populations are registered in Crop Science, they should be stored in the National Seed Storage

Laboratory (NSSL) at Ft. Collins, but not maintained in the working collection. All requests for such populations will be directed to the originating institution. Homozygous lines registered in Crop Science should be stored at NSSL and be available from the working collection. All germplasm releases would be reviewed by the Committee every ten years. Lines that are no longer useful and that have all progenitors in the collection would be eliminated from the working collection. It was suggested that Experiment Stations or other originating institutions be encouraged to assume more responsibility for maintaining seed of lines that they release. The committee will develop a policy statement and submit it to Crop Science and the National Plant Germplasm System for approval. Dr. Hartwig pointed out that information pertaining to germplasm releases and seed availability could be published as a note in Crop Science. Therefore, seed of these populations would have no requirement for storage.

Requests for PVP varieties will be sent to the originator. It was noted that policies are changing at public institutions and public PVP varieties will be studied further with regard to seed requests. Possibly a statement could be signed by the originating institution as to whether to restrict distribution through the originator or allow it to be available through the working collection. A list of accessions that were registered in Crop Science, but are not stored in NSSL or in the GRIN database, was reviewed. Originators of these missing cultivars, germplasm, parental lines, and genetic stocks will be contacted by the curator at Urbana IL to secure seed for storage at Ft. Collins.

The private variety collection has increased from five to 27. Requests will be formally made for more entries from various private companies.

2) **Evaluation Subcommittee:** The committee thought that everyone should know what is in the GRIN system by mailing a list of data available to Plant Sciences Research Departments and Private Companies to determine if there are gaps in the system that need attention.

The problem in evaluation is that large requests are filled but no data is returned relative to specific traits being evaluated. It was suggested that when germplasm is sent that any data relative to evaluation be returned within one year. This would include techniques used, where evaluation was conducted and include both positive or negative data for accessions evaluated. It was

mentioned that data, where appropriate, should be put in GRIN. Fatty acid or amino acid analyses should have high priority because of recent importance. Claudia Coble indicated a need to standardize data presentation through an official descriptor list. The descriptor list was reviewed and a few changes were made. Claude Coble made a motion (seconded by John Thorne) to approve the soybean descriptor list subject to review on a periodic basis. The motion carried. Problems with how to add new data or data from several different screenings was mentioned. It was pointed out that information in GRIN on quantitative traits is generally collected in one or two environments and may change with additional testing. Allan Stoner indicated that there was additional money available to add information to GRIN. People who have data that should be added to GRIN should contact Claudia Coble or Randy Nelson.

3) Operations Subcommittee: Claudia Coble indicated that new germplasm that is available for use will be updated yearly to the user community. Jim Orf suggested that the Soybean Genetics Newsletter could be an avenue to get newer collection releases publicized until a formal publication comes out.

Tommy Carter reported on his drought-tolerance collection trip to Southeast China in 1990. He was able to bring back seed from 30 accessions retrieved from areas low in pH and high in aluminum. Soil rhizobium samples were also collected from 22 sites in four provinces. He was not able to bring the soil or rhizobium back. The Chinese wanted research grants in exchange for soil samples and rhizobium. A motion was made that the Soybean CAC write a letter to support a proposal to fund a research exchange with the Chinese to allow us to receive seed, rhizobium, and soil from the areas sampled by Tommy Carter. The motion carried.

A variety evaluation of 28 cultivars exchanged between China and the US at seven locations was just completed. Data is in the process of being summarized.

Elections were held for the positions of Chairman and Vice-Chairman. Roger Boerma, University of Georgia, and Grover Shannon, Delta and Pine Land Company, Scott MS were elected Chairman and Vice-Chairman, respectively.

There being no other business, the meeting was adjourned.

James H. Orf, past chairman
Soybean Germplasm Crop Advisory Committee

Research Notes

AUSTRALIA

NSW AGRICULTURE AND FISHERIES

AGRICULTURAL RESEARCH AND ADVISORY STATION

PMB Grafton NSW 2460 Australia

1) Weathering tolerance in soybeans.

Introduction: Wet weather around soybean harvest time represents one of the more serious threats to grain yield and quality in many soybean production areas in Australia and in many overseas production areas, as well as reducing the quantity of acceptable quality sowing seed for the next season. In the past ten years significant losses were sustained in 1990, 1989, 1988, and 1983 in most Australian soybean areas, but has been more frequent in coastal and near-coastal areas in northern NSW and southeastern Queensland.

Prolonged rain after crop maturity and prior to harvest affects both yield and quality in soybean crops. Yield is usually reduced, in some instances by major proportions, through weight reduction in weathered seed and through less seed harvested as seed falls from pods opened by germinating seeds and badly weathered seed being blown out of the back of harvesters.

A project being conducted at Grafton (29° 37'S 152° 58'E) since 1988 has been looking at factors involved in soybean weathering, with the aim of producing more tolerant varieties. 'Dune' was registered in 1990 for coastal sowing (Desborough and Rose, 1990), based on superior weathering tolerance under both field and laboratory conditions.

Field Weathering: The degree of weathering of a range of genotypes in the field can be misleading if, at the time of the severe rain period, genotypes are at different stages of maturity. Immature soybeans will usually undergo only minor to no weathering. However, leaving mature plants in the field until a heavy rain period can be a useful guide. Table 1 shows the level of weathering in four soybean cultivars left in the field for post-harvest weathering in 1989, when all sites received prolonged rain during April and May. Order of maturity was 'Forrest' < 'Bragg' = Dune < 'Bossier'. Dune was more tolerant of these conditions than both earlier and later cultivars, a result supported by data collected in previous seasons (Desborough, 1988).

Laboratory Weathering: A rainfall simulator has been developed at Grafton that enables controlled weathering of whole, mature plants, collected from the field prior to any field deterioration. The simulator consists of a

chamber in which mature, dry plants are held upright and sprayed by a misting jet attached to a timing controller. Plants are sprayed for 10 secs each 1 min, which keeps the pods very wet. It has been found that sufficient weathering to separate tolerant from susceptible types has taken place after five days in the simulator.

Table 1. Percent unweathered seed sampled after post-maturity weathering in the field at three coastal sites in 1989, in soybean.

	Woodburn	Grafton	Wardell
<u>Date sampled</u>	<u>3 May</u>	<u>30 May</u>	<u>6 June</u>
Forrest	68	39	13
Bragg	78	64	39
Dune	91	84	74
Bossier	n/a	70	54

Forrest and Dune plants were collected from a field experiment after minor rain damage (<7% in any treatment). Field treatments included plus and minus fungicide (Benlate) for control of fungal diseases, particularly Phomopsis spp., which were applied biweekly until stage R3 and thence weekly until R8. After shed-drying, plants from each of the field replicates were placed in the simulator for 5 days and, after drying, seeds were assessed for weathering damage.

Results presented in Figure 1 show that fungicide reduced significantly the subsequent weathering in Forrest, but not in Dune. With the same fungicide treatment, Dune was significantly less weathered than Forrest. These results confirm the superior weathering tolerance of Dune measured in the field and also indicate the role fungal diseases, in all likelihood Phomopsis spp., are playing in the weathering complex.

The reasons for the greater weathering tolerance of Dune are not known, but studies are underway to determine which factor(s) impart this tolerance. Hardseededness does not develop in Dune. However, it is likely, as shown by a recent experiment at Grafton, that resistance to water entry by the pod may be one of these factors. Seed moisture content was measured for seeds collected sequentially from pods in the rainfall simulator and for seeds removed from the pod and placed on wet germination pads (Figure 2). Seeds of Dune and

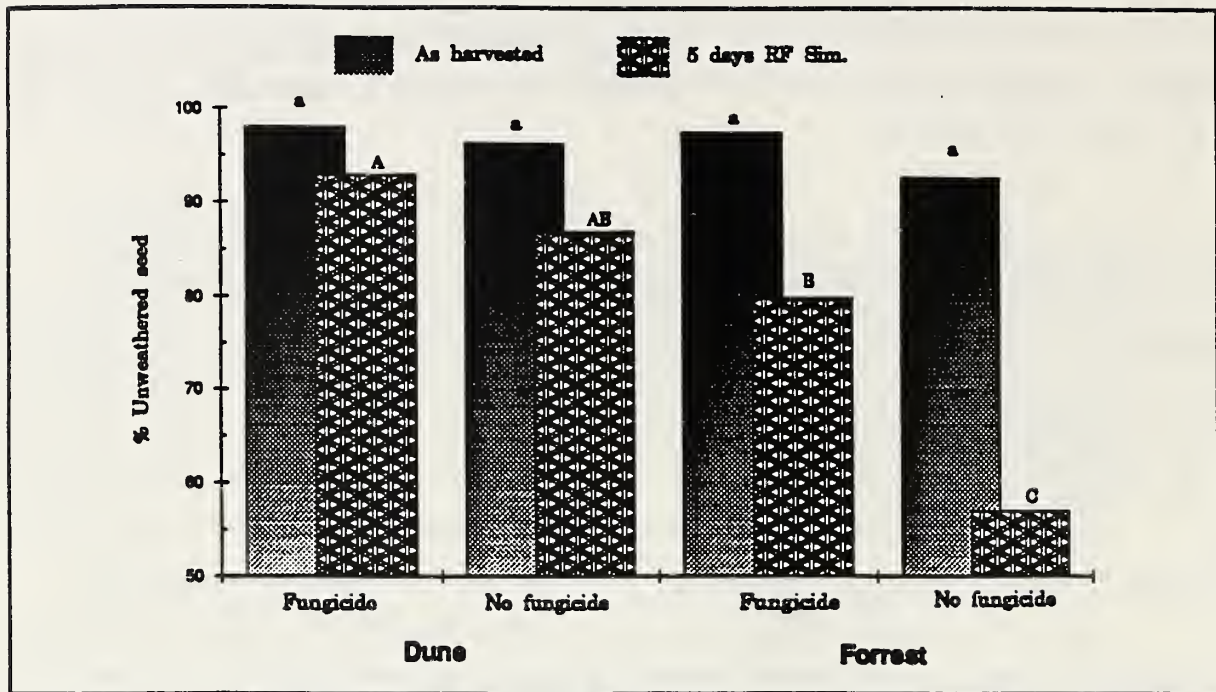


Figure 1. Effect of fungicide application in the field on weathering under rainfall conditions for two soybean cultivars.

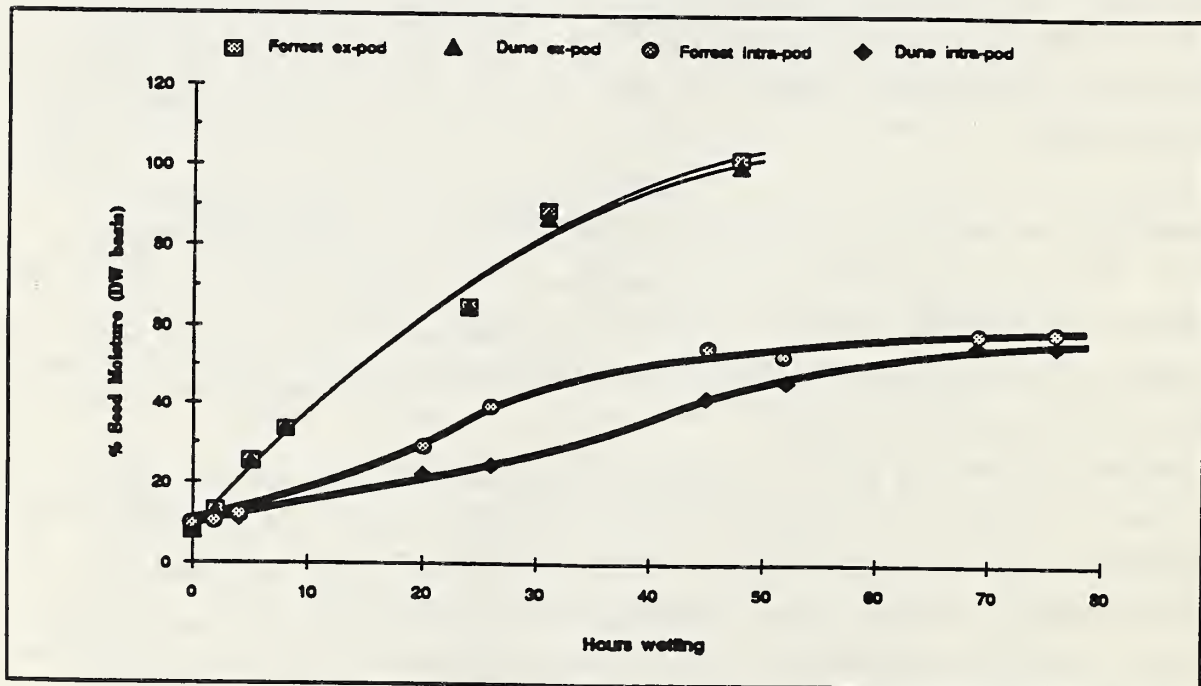


Figure 2. Seed moisture increase of Dune and Forrest soybeans when subjected to constant wet conditions either inside or outside the pod.

Forrest outside the pod increased moisture content at the same rate, but within the pod the rate of moisture increase was much slower in Dune than in Forrest during the first 2 days.

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Peter Desborough

INSTITUTE OF CROP GERMPLASM RESOURCES
CHINESE ACADEMY OF AGRICULTURAL SCIENCES
30 Bai Shi Qiao Road
Beijing, People's Republic of China

1) Imbibitional chilling injury in cultivars of soybean.

Effect of seed moisture and imbibitional temperature on germination and productivity was found during imbibition of soybean (Hobbs and Obendorf, 1972; Obendorf and Hobbs, 1970). Embryos of earlier maturing cultivars tended to be more susceptible to imbibitional chilling injury than those of later maturing cultivars (Bramlage et al., 1979). Early maturing varieties with the ability to form pods and seeds at lower temperatures were most susceptible to irreversible injury during imbibitional chilling and seeds that were most susceptible to imbibitional chilling also had the highest rate of water uptake (Orr et al., 1983). Soybeans with black pigmented seed coats, which showed less vigor loss following imbibition at 0°C, imbibed water more slowly than nonpigmented soybeans (Tully et al., 1981). The period of profuse leakage is interpreted as a time of membrane reorganization, and imposing a low temperature during this period prolongs the rapid leakage, suggesting delayed or faulty membrane reorganization (Bramlage et al., 1978).

After our studies on screening in the laboratory in 1987 and 1988 (Li et al., 1989) and appraisal in the field in early spring in 1989 (Li et al., 1990) for cold tolerance of soybean, we have used water uptake and solute leakage to assess the mechanism of chilling injury during imbibition of soybean in the present study and found some results supporting Bramlage's (1978), Tully's (1981) and Orr's (1983) findings.

Materials and Methods: The following materials used in the present experiment were chiefly reaped from emergence in the field in early spring in 1989 (Li et al., 1990).

Seeds used for germination test at low temperature of 6°C were sterilized in advance according to a preceding report (Li et al., 1989). The seeds were directly placed in a chamber at 6°C for 14 days, with three replications without light. A paired comparison design was conducted with seeds imbibed at

ambient temperature of 27-29°C for 8 hr and then placed at 6°C for 14 days.

After the seeds were treated at 6°C for 14 days, they were transferred into the field for emergence trials compared in pairs with normal emergence of dry seeds.

Soybean seeds were put in beakers with 30 ml distilled water, then placed in chambers at 6°C and 25°C, separately, for some time, with three replications in order to measure water uptake and electrical conductivity. The imbibed seeds were weighed before (W1) and after (W2) toasting at 95°C in an oven for 24 hours. Water uptake (WU) was calculated according to the following formula:

$$WU = (W1 - W2) / \text{No. of seeds}$$

The solution of seeds soaked in distilled water in the beakers was measured at ambient temperature to determine electrical conductivity (EC) with a conductometer model DDS-IIA made in Shanghai, People's Republic of China.

Results and Discussion: Germination and imbibitional chilling injury-- Germination percentage of B8 and B5 imbibed directly at 6°C for 14 days was the lowest among nine cultivars (Table 1) from emergence tests in the field at low temperature conditions in early spring in 1989 (Li et al., 1990), which were conducted as cold-susceptible genotypes and others were cold-tolerant ones, due to their higher percentage of germination. Percentage of germination of the nine cultivars imbibed at low temperature of 6°C was no more than 50% (Table 1), indicating that soybean seeds were damaged fatally during imbibition at low temperatures. However, the cold injury during imbibition was alleviated by imbibing soybean seeds at ambient temperatures of 27-29°C for some time. For example, germination percentage of B1 imbibed at normal temperature for eight hours in advance and moved into 6°C was 65.5%, compared with 34.5% for those imbibed at 6°C directly. The paired experimental design of eight cultivars but B7 also manifested that germination percentage of soybean seeds imbibed at normal temperatures was significantly higher than those imbibed at low temperature. Thus, this explains that soaking soybean seeds in water at ambient temperature before sowing mitigates the chilling injury in the field in early spring and during cold seasons.

Emergence and imbibitional chilling injury--After imbibing at 6°C for 14

days, emergence percentage of B7, B6, B9, B3, B2, B4, and B1 in the field were 28%, 25%, 14%, 13%, 12%, 11%, and 10%, respectively, which showed that these cultivars were more tolerant than B5 and B8 cultivars, which did not emerge in the field (Table 1). The percentage of emergence of all nine cultivars was less than 50%, and percentage of germination (Table 1) demonstrated that soybean seeds suffered serious chilling injury during imbibition at a low temperature and that the injury affected their emergence in the field greatly. Besides, emergence percentage of dry seeds sowed in the field was significantly different or much higher than that of seeds imbibed at low temperature of 6°C for 14 days. Soybean seeds with green seed coats (e.g., B8 and B5) displayed easier injury and intolerance to cold during imbibition because of the loss of their germination and emergence (Table 1).

Table 1. Germination, emergence, leakage and water uptake of soybean seeds imbibed at 6°C.

Cultivars	Germination (%)	Emergence (%)	WU(l)	EC(i)
B1	44.9	10.0	206.25	36.50
B2	42.0	12.0	193.53	22.63
B3	35.0	13.0	25.23	3.40
B4	21.9	11.0	193.82	18.83
B5	6.7	0.0	377.03	50.17
B6	16.7	25.0	63.37	8.25
B7	48.3	28.0	165.27	12.78
B8	6.7	0.0	412.83	61.67
B9	19.5	14.0	40.90	3.33

l = WU-mg/seed

i = EC-uv/cm seed

Water uptake of different cold-tolerant genotypes--Significant and negative correlation between water uptake and relative germination ratio was found in soybean imbibition tests and germination tests at 6°C and 25°C, respectively (r 6°C = -0.475**, r 25°C = -0.417**). The more water per seed absorbed, the lower relative germination ratio of its cultivar, which was considered as more susceptible to cold. Water uptake was one of the criteria of cold-tolerance.

Water uptake of B3, B9 and B6 cultivars, with black seed coats, was

lower (Table 1) when imbibed at 6°C for 24 hours, which indicated their tolerance to cold. On the contrary, water uptake of B8 and B5 was highest, indicating their intolerance to cold. B7, B2, B4, and B1 cultivars were located in the mid-range of water uptake, indicating mid-tolerance to cold. This partly supported results of emergence tests in the field in the early spring (Li et al., 1990). B8 and B5 cultivars showed cold-intolerance, also supporting the results of germination and emergence tests described above.

Electrical conductivity and mechanism of cold tolerance--Cell membrane permeability reflects on seed electrical conductivity. Electrical conductivity correlated negatively with relative germination ratio at different temperatures in soybean during imbibition significantly (r 6°C = -0.4568**, r 25°C = -0.522**). The lower electrical conductivity per seed, the more tolerance to cold. Positive correlation between electrical conductivity and water uptake at 6°C and 25°C (r 6°C = 0.823**, r 25°C = 0.819**) remarkably expressed the increase of solute leakage from soybean seeds following water uptake during imbibition. Therefore, electrical conductivity can be suggested to be a criterion of tolerance to cold, just like water uptake.

Electrical conductivity of B9, B3, B6, and B7 rehydrated at low temperature of 6°C was lower, which showed that the cultivars were more tolerant to cold, as contrasted with B8 and B5, whose electrical conductivity was the highest among nine cultivars. B4, B2, and B1 cultivars were in the mid-range (Table 1). It is thus clear that the trend of the electrical conductivity of nine cultivars is in accordance with the water uptake, shown above.

The present experiment also demonstrated that the period of rapid electrolyte leakage from seeds was prolonged by imposing a low temperature and that intolerance to cold of some seeds from emergence tests in early spring (Li et al., 1990) had some changes by the selection of low temperature conditions in the field.

Electrical conductivity (Fig. 1) of soybean seeds imbibed at 6°C went up to the top rapidly within two hours, then reduced. Membranes were possibly reorganized from leakage and dehydration at this period. Electrical conductivity declined more rapidly from two to four hours, then slowed more gently after imbibing at low temperature for four hours. Membrane reorganization was going on, preventing marked leakage at that time. The speed

of membrane reorganization in cold-susceptible genotypes (e.g., B8) which was damaged more easily, was less, from leakage and dehydration than that in cold-tolerant ones (e.g., B2) at low temperature, for electrical conductivity of cold-intolerant cultivars (e.g., B8) was greater than that of more cold-tolerant ones (e.g., B2) every time. The reduction of electrical conductivity of cold-intolerant cultivars (e.g., B8) was slower than that of cold-tolerant ones after imbibition at low temperature of 6°C for two hours, especially from two to four hours.

Conclusion: Cold injury of soybeans during germination occurred in early imbibition at low temperatures.

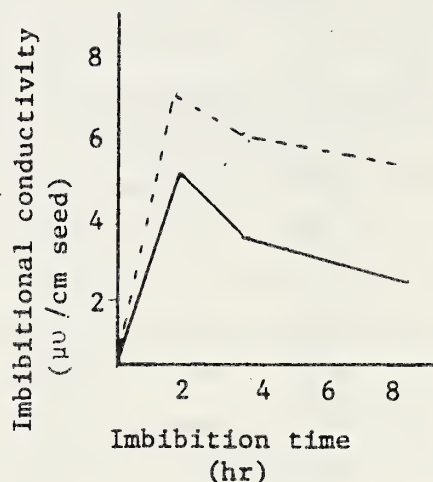


Figure 1. Electrical conductivity of soybean seeds imbibed at 6°C for different times

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Li Yujun

Chang Ruzhen

Zhao Yutian

2) The effect of polyethylene glycol and ascorbic acid on soybeans for cold tolerance.

The vigor of peanut seeds conditioned with 20% polyethylene glycol (PEG) at 10-15°C for 8 hours was raised and the activities of catalase and superoxide dismutase (SOD) in seeds treated with PEG were higher than that in controls after germination for three days (Chen and Fu, 1987). The seedlings of two cucumber cultivars with different cold tolerance exhibited some differences in both SOD activity and leakage of electrolyte (Liu et al., 1985), as well as soybean seeds of different cold-tolerant genotypes imbibed at low temperature (Li, 1990b). The ascorbate content in rice seedlings decreased with the time of the exposure to chilling temperature, which was inhibited in rice seedlings pretreated with antioxidants glutathione, L-cysteine and mercaptoethanol (Zeng et al., 1987).

In the present study, the treatment of polyethylene glycol and ascorbic acid was used for assessing the effect on leakage, superoxide dismutase, and malondialdehyde (MDA) of soybeans for tolerance to cold.

Materials and Methods: Four cultivars of soybean with different tolerance to cold were harvested in 1989 from emergence tests in early spring (Li et al., 1990a) and then were tested. Soybean seeds rinsed in sterilized

distilled water were soaked in 10% PEG-4000 at 6°C for several days, as well as in 5% ascorbic acid.

The hypocotyls of the seeds were thoroughly ground in a cold mortar and pestle in an ice bath, until no fibrous residue could be seen. The grinding medium (40 ml/g fresh weight) was 0.05 M Na-phosphate buffer (pH 7.8). The homogenate was centrifuged at 14,000 g for 10 min at low temperature. The supernatant, hereafter referred to as crude SOD extract, was used for electrophoresis and for determination of SOD activity and formation of MDA.

Polyacrylamide gel electrophoresis of the crude SOD extracts was performed according to Luo and Wang (1983).

The superoxide dismutases were localized by the photochemical procedure of Beauchamp and Fridovich (1971). The stained gels were scanned with a Shimadzu Dual-wavelength Chromatogram Scanner Model CS-910.

Activity of SOD was measured according to Steward and Bewley (1980). The content of malondialdehyde (MDA), a product of membrane-lipid peroxidation, was determined by the method of Lin et al. (1984).

Soybean seeds were soaked in 5% ascorbic acid at 6°C for 48 hours and then rinsed in distilled water for germination at 20°C for 51 hours, again rinsed in distilled water for further germination at 10°C for 6 days, while emergence testing in the field was conducted with the same treatment until germination at 10°C for a day.

Results and Discussion: The electrical conductivity of B8 seeds soaked in 10% PEG-4000 dropped doubly, as compared with that of B8 seeds soaked in distilled water at low temperature of 6°C for 5 days (Table 1). On the contrary, the electrical conductivity of B8 seeds soaked in 5% ascorbic acid increased markedly, when compared with that of B8 seeds treated in control, at low temperature.

The area of SOD activity bands by scanning of activity band on electrophoresis of B8 hypocotyls soaked in 10% PEG-4000 increased markedly more than that of B8 hypocotyls treated in control (in distilled water), with its opposite of that treated in an ascorbic acid solution, at low temperature of 6°C (Table 1).

The change of SOD activity of soybean hypocotyls of B8 soaked in a PEG or an ascorbic acid solution was similar to the electrical conductivity of B8 seeds above, in contrast with the change of MDA content of B8 hypocotyls soaked in a PEG or an ascorbic acid solution, compared with those of B8

treated in control (Table 1).

The electrical conductivity of the seeds of B2 cultivar soaked in 10% PEG-4000 at low temperature of 6°C, as well as the area of SOD activity bands, the activity of SOD and the content of MDA, also differed from that soaked in 5% ascorbic acid at 6°C (Table 1) for 6 days. The trend of the changes in B2 cultivar was in accordance with that in B8 cultivar. It was evident that cold tolerance in soybean seed osmosed with a PEG solution during germination at low temperature of 6°C was enhanced, for their electrical conductivity decreased in company with the reduction of SOD activity and the increases of the content of MDA and the area of SOD activity bands, and that cold tolerance in soybeans soaked in an ascorbic acid solution during germination at low temperature was decreased.

Table 1. Effect of PEG-4000 (10%) and ascorbic acid (5%) on leakage, SOD and membrane-lipid peroxidation in soybean.

Cultivar	Treatment	Electrical conductivity (i)	Area of SOD activity bands	Sod activity (j)	MDA content (k)
B8	Soaked in distilled water (control) 6°C for 5 days	3700	39,734	53.9	188.3
	Soaked in 10% PEG-4000 6°C for 5 days	1540	202,484	31.4	203.4
	Soaked in 5% ascorbic acid 6°C for 5 days	3860	9,106	70.3	141.5
B2	Soaked in 10% PEG-4000 6°C for 6 days	1150	101,864	95.5	107.8
	Soaked in 5% ascorbic acid 6°C for 6 days	2360	76,143	143.4	99.3

(i) = uv/cm; (j) = Unit/g fr.wt.; (k) = u mol/g fr.wt.

After soybean seeds imbibed at normal temperature for eight hours, no significant difference of germination percentage exhibited between the seeds soaked in 10% PEG (58.9%) and that soaked in distilled water (59.5%). This suggested not only that no evident function of PEG osmosis existed after soybean seeds imbibed at normal temperature for eight hours and the cold-

tolerance of soybean seeds may be heightened at low temperature if they are treated with a PEG solution at ambient temperature before imbibition, but also that the cold injury of soybeans during germination occurred in early imbibition was supported.

The enzyme band of Mn-SOD (a) in soybean hypocotyls reduced when their seeds were soaked in a 5% ascorbic acid solution at low temperature of 6°C (Fig. 1), suggesting possibly the injury of plasma membrane related to the reduction of Mn-SOD band and the increase (Table 1) of leakage of electrolytes.

The percentage of germination in B2, B4, B7, and B8 cultivars soaked in 5% ascorbic acid was less than that soaked in distilled water, as was the percentage of emergence (Table 2). Soybean seeds were damaged more seriously at low temperature when soaked in 5% ascorbic acid, suggesting faulty membrane reorganization and cold-tolerance decreased during germination. The percentages of germination and emergence of B7 cultivar were higher than those of the others whenever soaked in distilled water or an ascorbic acid solution (5%), which meant that a more cold-tolerant mechanism existed in soybean seeds of B7 which was selected by low temperature conditions in early spring in 1989 in the field (Li et al., 1990a).

Mn-SOD inhibited and reduced whether by an ascorbic acid or the pH of the ascorbic acid solution may be continued.



Figure 1. Scanning of electrophoretic SOD band of hypocotyls of B8 cultivar imbibed at 6°C for five days in soybean.

CK-soaked in distilled water; A-soaked in 10% PEG-4000; C-soaked in 5% ascorbic acid.

Table 2. Germination and emergence of soybeans soaked in 5% ascorbic acid at low temperature of 6°C for 48 hours.

	<u>Percentage of germination</u>		<u>Percentage of emergence</u>	
	Ascorbic acid	Distilled water	Ascorbic acid	Distilled water
B2	20	55	0	12
B4	20	50	0	24
B7	35	75	13	28
B8	20	25	0	4

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Li Yujun

Zhao Yutian

Chang Ruzhen

SOYBEAN RESEARCH INSTITUTE HAAS

Harbin 150086

People's Republic of China

1) Waterlogging resistance and peroxidase activity in soybeans.

Peroxidase in the plant body can be atalysis to reduce toxic substance and make the plant free from its injury (Cao Zongxun, 1980). There is a relationship between drought resistance of soybeans and their peroxidase activity (Song Yingshu, 1986). Is there some relation between waterlogging and peroxidase activity of soybean? This program was to study this kind of relationship and to look for methods of measuring waterlogging resistance in reference to soybean production and germplasm of waterlogging resistance.

Materials and Methods: Eight soybean varieties (Hefeng 22, Hefeng 25, Fengshou 12, Heinong 16, Heinong 26, Baofeng 2, Dongnong 82-833, and Ke 8118) were cultivated in pots soaked with water from V3, R1, and R3 stages, separately, for 20 days and until maturity, in 1988 and 1989. Activity of peroxidase was measured every three days, from the second to the eighteenth day after soaking began. Controls were their relevant varieties, unsoaked. The fourth leaf opened fully, counted from the top of the plant, was sampled to measure the activity of peroxidase, by the method of Huadong Teachers' University. Main agricultural characters and yield per plant were measured at maturity.

Results and Analysis: 1. Changing of peroxidase activities of soybeans-
-When soybean plants were soaking with water from R1 stage, the activities of peroxidase were increasing gradually from day 2 to day 11. The highest value was at day 11. Then the activities reduced quickly. Figure 1 shows that the values of peroxidase of varieties soaked were lower than that of the check. From day 5 to day 18, the difference of peroxidase activities between soaked plants and corresponding control was significant (Table 1). This meant that injury of waterlogging can be known by the activity of peroxidase.

Peroxidase activities were different among the varieties soaked with water. This kind of difference was more significant from day 5 to day 11 after beginning soaking. If the activities were measured earlier than day 5,

or later than day 11, the difference among the varieties was not significant.

Table 1. Significance of difference of peroxidase activity between soaked plants and control, in soybeans.

Days soaked with water	2	5	8	11	14	18
Mean square deviation	0.000	0.027	0.026	0.140	0.035	0.027
F-value	0.01	8.85*	51.94**	78.67**	6.48+	10.81*

+, *, ** = significant at 0.10, 0.05, and 0.01 levels, respectively.

2. Relationship between peroxidase activity and waterlogging injury of soybeans--The relationships between peroxidase activity and waterlogging injury of soybeans soaked from different growing and developing stages were studied. The results showed that this kind of relation was not significant when soaking began from V2 or R3 stages ($r = 0.183$ and $r = -0.172$, respectively). But the correlation was significant when soaking began at the R1 stage; i.e., the waterlogging resistance of soybean varieties can be known by measuring activity of peroxidase of plants soaked with water at the R1 stage.

Data in Table 2 show that when soaking began in the R1 stage, there were significant correlations between peroxidase activity and main agricultural characteristics in the eleventh to eighteenth soaking day. But plant height, number of branches, and 100-seed weight would be exceptions.

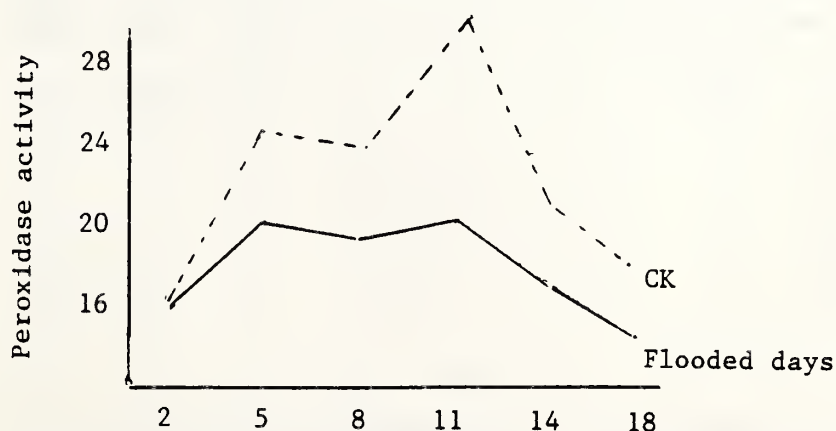


Figure 1. Peroxidase activity of soybeans soaked with water from R1.

3. The difference of waterlogging resistance among soybean varieties-- After water soaking, there was a significant difference of yield and yield factors of soybeans (Table 3). It meant that there was a difference of waterlogging resistance among soybean varieties. Baofeng 2 showed high plant, more effective nodes, more pods, more seeds per plant, bigger seeds and highest yield (5.87 g/plant). It is a waterlogging-resistant variety. Ke 8118 showed less effective nodes, fewer pods and seeds per plant, and the lowest yield (2.57 g/plant). It is a waterlogging-unresistant variety. Their peroxidase activity was 21.01 D470/minute.gram of fresh weight and 19.10 D470/m.g, respectively.

Table 2. Correlation between peroxidase activity and main agricultural characters of soybeans soaked from the R1 stage.

Days soaked	Plant height	No. of nodes	No. of branch	Wt. of plant	Seeds per plant	3-4 seed pods	Seeds per plant	Yield per plant	Wt. of stem	Wt. of 100 seed
2	-0.29	0.72+	0.22	0.76+	0.80+	0.69	0.57	0.78+	0.69	-0.29
5	-0.16	0.65	-0.22	0.65	0.50	0.54	0.45	0.61	0.66	0.11
8	0.05	0.64	-0.41	0.57	0.30	0.43	0.32	0.58	0.51	0.35
11	0.34	0.80+	-0.21	0.85*	0.67	0.82*	0.72+	0.87*	0.76+	-0.04
14	0.57	0.51	0.17	0.71	0.77+	0.89*	0.86*	0.74+	0.63	-0.51
18	0.55	0.81*	-0.07	0.88*	0.72+	0.88*	0.86*	0.94*	0.73+	-0.22

* = significant at the 0.05 level; + = significant at the 0.10 level.

Table 3. Main agricultural characteristics of soybeans soaked with water.

Variety	Plant height	No. of nodes	No. of branch	Pods per plant	3-4 seed pods	Wt. of plant	Yield per plant	Seeds of plant	Wt. of 100 seeds	Wt. of stem
Ke 8118	65.4	11.5	0.2	8.1	3.3	8.1	2.6	18.0	15.1	5.6
NF 12	52.1	12.2	0.9	11.2	6.3	8.8	3.3	27.3	12.0	5.6
HF 22	57.6	11.6	1.6	11.4	3.8	10.1	3.5	23.9	15.7	6.6
HF 25	60.3	13.7	0.1	13.1	4.8	10.6	4.2	27.5	15.4	6.4
HN 16	67.9	14.2	1.7	13.7	6.0	10.7	4.1	30.1	13.9	6.6
HN 26	70.5	13.7	1.1	14.0	5.2	11.9	4.1	29.7	13.6	7.6
DN 82833	67.4	14.4	2.0	16.4	5.3	12.4	4.8	32.8	13.1	7.6
BF 2	77.5	14.8	0.9	13.1	8.4	14.1	5.9	33.8	16.8	8.1
F-value	1.8	4.5*	4.4**	2.7*	4.5**	2.3*	2.9*	0.7	1.1	0.7

* = significant at the 0.05 level; ** = significant at the 0.01 level.

NF = Nenfeng; HF = Hefeng; HN = Heinong; DN = Dongnong; BF = Baofeng.

Conclusion: In the condition of soaking, peroxidase activity of soybeans was reduced by different degrees, as variety genotypes. When soybeans were soaked with water from the R1 stage, there was significant correlation between peroxidase activity and yield in the period of day 11 to day 18; thus waterlogging resistance of soybeans can be known by measuring activity of peroxidase at soaking condition. Because the period in which there was significant difference among varieties of soybean was from day 5 to day 11 soaked from R1, the day best suited to measure waterlogging resistance by activity of peroxidase was day 11. And the earliest day for that was not earlier than day 8; the latest day was not later than day 14.

Baofeng 2 is a waterlogging resistant variety. Ke 8118 is a waterlogging susceptible variety.

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Xu Yonghua
 He Zhihong
 Song Yingshu
 Du Zhiqin

HEILONGJIANG ACADEMY OF AGRICULTURAL SCIENCES

50 Xuefu Road, Harbin 150086

The People's Republic of China

1) Preliminary study on γ -rays chronic radiation for growing plants in soybean.

In order to increase genetic variance of soybean and find a new way for soybean mutation breeding, the effect of gamma rays chronic radiation for growing plants in soybean has been studied since 1987.

Materials and Methods: Ha83-1, PI 423948A and 'Ozzie', composed of pure lines or varieties were treated with exposure doses of 6, 8, 10, 13, 30, 50, 70, and 100Gy and dose rates of 0.07, 0.09, 0.17, 0.19, 0.29, 0.50., 0.84, and 1.45 rad/min of gamma rays. Plants were grown in pots and were radiated at VE-V1 and R1-R2 stages, 112 plants in eight pots, per treatment.

A week after radiation, we began to record seedling height, variance type, the number of pods per plant, and seed size. The total M_1 seeds were planted in the field for the M_2 population. In the M_2 - M_3 generation, 10-50% of the plants were selected to be tested and were grown for the following generation in the next year. Each treated population included at least 1000 plants.

Results: 1) Radiation at VE-V1 stage. The studies indicated that growth of plants was inhibited significantly in the M_1 generation of PI 423,948A. Growth rate of plants in every treatment was lower than that in the untreated control five weeks after radiation; the higher the dose, the lower the growth rate. The exposure dose of 50Gy was the critical dose for growing plants irradiated, in soybean. The studies also showed that plant growth was inhibited more seriously under a lower dosage rate than under a higher dosage rate with the same dose. Seventeen days after treatment, dry weight accumulation was increased in the 50Gy group due to the plant development being hindered. There were morphological changes in the M_1 generation, such as flat stem, 2-4 growth points or curving stem (360°), with 30Gy radiation. The seventh or eighth trifoliolate leaves of the main stem in plants were observed to have one, two, or four leaflets--the frequency was very high. This phenomenon hadn't been found in the seed radiation population. In the M_1 generation, sterile plant percentage increased and the number of pods per plant was reduced. This occurred in the 50Gy group much more than in the 30Gy

group ($p < 0.01$) (Table 1).

In the M_2 generation, some morphological changes, such as one or three cotyledons, unifoliolate leaves, foliation cotyledon and linked cotyledons at the VE stage were noted. The frequency of induced mutation tended to increase as the dosage became stronger and the dose rate became lower. The means of seed weight per plant in every treatment was higher than untreated control ($p < 0.05$ or 0.01), ranging from +35.8 to +58.7%. There were 5-23.8% plants whose per plant seed yield increased 83.5% over untreated control. Under the same dosage treatment, the frequency of high yield per plant in the dosage rate of 1.45rad/min group was higher than that in the 0.50rad/min group.

Coefficient of variation in seed size and plant height increased with different ranges. For example, the weight of 100 seeds in the M_2 of 'Ozzie' was significantly greater than that of control ($p < 0.05$ or 0.01). In the M_3 , we found 20.4% of the plants showed a seed yield increase of 30% over untreated control. Seed yield per plant ranged from 7.2 to 47.0 grams; the control ranged from 7.0 to 27.2 grams.

2) Radiation at the R1-R2 stages. Radiation damage to plant growth, fertility, and so on, tended to increase as the dose became higher. The treatment with a lower dosage rate produced more serious damage. Plant height and growth rate were reduced and differences were significant among treatments ($p < 0.01$). Flowers radiated at the differentiation period couldn't form pods and seeds, the pollinated flower also couldn't form seeds in all. Among them some flowers set sterile pods or pods with one seed. Treated plants had fewer pods than did controls. The number of sterile pods increased as dosage became higher, sterile pod rate reached 50% or more in the 50 Gy treatment group. Treating plants at three periods--the flower bud stage (7-14 days before flowering), the zygote-proembryo stage (0-2 days after flowering), and the young embryo stage (10-20 days after flowering)--demonstrated that the group treated at 0.2 days after flowering had higher sterile pod percentage in the M_1 than the group treated at 10-20 days after flowering. But the pod-setting percentage of those treated at the zygote stage was lower than those treated at the young embryo stage (Table 2).

The treatment with 30 Gy resulted in larger seed size and smooth seed coat; the treatment with 50 Gy resulted in smaller seed size and cracked seed coats.

Table 1. Characters of PI 423,948A in M_1 after treatment at VE-VI stage.

Dose (Gy)	Dose rate (rad/ min)	Growth rate		Dry weight		Pods per plant	Weight per 100 seeds	Plant height (cm)
		(cm/day)		17 days				
		days after		after				
		<u>radiation</u>		<u>radiation</u>				
		7-17	17-35	mg/cm	% of ck			
30	1.45	1.51	2.43	65.0	90.2	30.1	12.9	102.9
	0.50	1.23	2.53	67.3	93.4	33.4	13.4	106.3
50	1.45	0.54	2.11	83.5	116.0	18.2**	13.7	99.8
	0.50	0.31	0.91	99.5	138.2	9.8**	13.8	39.4
control		1.51	3.53	72.0	100.0	41.7	14.2	110.4

Table 2. Effect of radiation on pod setting characters of soybean in the M_1 generation (1987, Harbin).

Dose (Gy)	Dose rate (rad/min)	Treatment stage	One seeded pods(%)	Sterile pods(%)	No. of seed/pod	100 seed weight
30	1.45	flower bud	57.4	6.6	1.3	24.5
		original embryo	56.4	18.2	1.2	20.7
		young embryo	22.9	0.7	1.6	20.6
50	1.45	flower bud	72.6	15.7	0.9	16.5
		original embryo	40.8	36.8	0.8	16.6
		young embryo	33.0	11.4	1.9	15.9

In the M_2 generation, frequency of morphological aberrations of seedlings after radiation treatment at the blooming period was greater than those radiated at the seedling period. Observation was made that different dose rates produced different results; e.g., 27.2% morphological abnormalities in the progenies radiated with 30Gy 1.45 rad/min vs. 40.7% with 30Gy 0.50 rad/min. Abnormal plant rate increased as the dose went up and dose rate went down. For example, abnormal plant rate was 67.7% in the treatment with dose rate of 0.50 rad/min, but 34.6% with 1.45 rad/min in the same dose of 50Gy; semi-sterile plant rates were 9.2% and 12.9%, respectively.

Plant height, seed weight per plant, and weight of 100 seeds were changed. Coefficient of variation and range on seed weight per plant in three treatments under the dose rate of 1.45 rad/min were larger than control. There were 3.89% plants whose seed weight per plant was twice as high as that of the control (Table 3). In the M_4 , we tested seed yield of 26 lines of

Ha83-1 and found 5 lines with a yield more than that of the control. Frequency of seed size was significant. Ozzie radiated with 15Gy 1.45 rad/min had mutants with large seed size in the M_2 and that remained 24.4-26.0 grams per 100 seed in the M_3 , the control with 16.1 grams per 100 seeds. Plant height, maturity, seed color and hilum color were the same as the control.

Table 3. Agronomic characters of soybean plants in the M_2 generation (1988, Harbin)

Dose (Gy)	Dose rate (rad/min)	Treatment stage	No. of plants	Plant height (cm)	Seed weight/plant(g)	Range of total weight(g)
control			30	125.7	20.7	13.0-37.3
1.5	0.5	flowering	208	133.6	20.3	8.1-44.4
30		plant	176	109.6	20.4	7.0-50.4
50			13	117.1	29.0**	18.6-52.4
1.5	1.45		222	126.0	23.3**	6.8-50.8
30			288	122.4	23.6**	5.0-54.9
50			102	122.3	22.9**	5.4-52.6
30	1.56	flower bud	31	108.0	25.1*	2.9-56.4
		original embryo	54	122.3	26.6**	6.7-47.8
		young embryo	81	121.2	22.2	6.6-52.8
50	1.56	flower bud	10	113.6	27.1*	11.9-44.8
		original embryo	11	112.8	28.1*	14.5-44.2
		young embryo	31	108.0	25.8*	4.8-61.4

On the basis of six replication experiments from the M_1 to M_4 generations, the conclusion was that gamma ray chronic radiation for growing soybean plants can increase the coefficient of variation and extend the range of variation on 100-seed weight and seed size, as well as seed yield per plant. For high yield breeding, the suitable treatment should be doses of from 30 to 50 Gy and dose rate from 0.50 to 1.45 rad/min at the seedling stage, or doses from 15 to 50 Gy and dose rate of 1.45 rad/min at the blooming stage.

This study provided a possible method for soybean mutation breeding, but it was carried out for only four years, and will be continued in the future.

Wang Peiying

Yu Baishuang

2) Study on soybean recurrent selection applied to male-sterility.

a) Effect of parents and their placing pattern on natural crossing: It has been observed that recurrent selection is effective to enrich gene resources, but it is very difficult to realize due to the limit in number of seeds crossed. For combinations with male steriles, a lot of crossing must be done. This experiment was designed for seeking a better method to produce seeds and for increasing natural crossing.

Materials and Methods: In 1988, two cross combinations were made with ('Wayne' msms x 'Calland') F_3 and (Calland msms x N.K) F_3 as female parent and 135 varieties/breeding lines developed in Heilongjiang Province as mass male parents; one combination with (Calland msms x N.K) F_3 as female parent and 70 varieties/breeding lines developed in the USA as male parents. Female parents were grown intermixed with male parents in the fields of the Soybean Research Institute, Heilongjiang Academy of Agricultural Science. Thirty plants were randomly sampled for each combination to accumulate pod and seed numbers.

Table 1. Group size of different combinations and placing patterns.

Combination	Com. no.	Male:female	Total rows (4m/row)
(Wayne <u>msms</u> x Calland) F_3 x L.J*	88-III	3:1 mixed#	15
(Calland <u>msms</u> x N.K) F_3 x L.J	88-IV	3:1 mixed	32
Calland <u>msms</u> x U.S.Mass	88-V	2:5 mixed	10
(Wayne <u>msms</u> x Rampage) F_4 x L.J	89-Ia	2:1 alternated^	17
(Wayne <u>msms</u> x Rampage) F_4 x L.J	89-Ib	2:1 mixed	17
(Calland <u>msms</u> x Rampage) F_4 x L.J	89-IIa	2:1 alternated	20
(Calland <u>msms</u> x Rampage) F_4 x L.J	89-IIb	2:1 mixed	16
Rampage <u>msms</u> x Heinong 34	89-IIIa	2:1 alternated	14
Rampage <u>msms</u> x Heinong 34	89-IIIb	2:1 mixed	16
(Wayne <u>msms</u> x Rampage) F_4 x Heinong 33	89-IVa	2:1 alternated	17
(Wayne <u>msms</u> x Rampage) F_4 x Heinong 33	89-IVb	2:1 mixed	16

* L.J = 135 or 140 varieties bred in Heilongjiang Province, mixed.

Mixed means female parents intermixed with male parents.

^ Alternated means female parent rows alternated with male parent rows.

In 1989, four combinations were made with (Wayne msms x 'Rampage')F₄, (Calland msms x Rampage)F₄ and Rampage msms as female parents and 148 varieties/breeding lines developed in Heilongjiang Province as mass male parents, Heinong 33 and Heinong 34 as single male parents. Proportion of female/male is equal to 2:1 and placing pattern included one row female parent alternated with two rows male parent, or female parent intermixed with male parent. Thirty plants were randomly sampled for each combination to accumulate pod numbers.

Results and Discussion: Parental influence on effect of crossing ability. Male parents in combinations 89-I and 89-II were the same (L.J mass parents) and female parents were different. A different number of pods was gotten from them. Female parents of the combination 89-II (Calland msms x Rampage)F₄ matured earlier than the female of combination 89-I (Wayne msms x Rampage)F₄, so the result was controlled by synchronism of flower period of parents and the affinity between parents. However, different pod number existed still in combinations of 88-III and 88-IV even though they had the same male and different female parents, with similar flowering period. It is clear that genotype influence was important in natural crossing.

Table 2. Parental influence on effect of natural crossing.

Combination number	Pod no. per plant	Seed no. per plant	Combination number	Pod no. per plant
88-III	7.30	10.10	89-I	16.35
88-IV	8.45	11.55	89-II	19.45
88-V	10.80	15.05	89-IV	17.55

Combinations 89-I and 89-IV had the same female parent, later maturing (Wayne msms x Rampage)F₄. Pod number of combination 89-IV with male parent of Heinong 33 was more than that of 89-I with male mass parents (148 L.J varieties). The longer flowering period of mass parents, in which there were the earlier and later maturing plants than Heinong 33, favored the female parent meeting male parents in flower, and increasing the percent of pollination. However, pod number of 89-I was less than that of 89-IV. Combination 88-IV, in which the

male was L.J mass parents, had the same female as combination 88-V, in which the male was U.S. mass parents. The former flowering period was longer than that of the latter, but 88-V was better than 88-IV in producing seeds from natural crossing. So parent influence was much more important than synchronization of parent flowering for producing a large number of seeds from natural crossing (Wayne msms x Rampage) F_4 crossed either with single parent (89-IV) or with mass parents (89-I), had a pod number that was less than (Calland msms x Rampage) F_4 (89-II) and Rampage msms (89-II). This indicated a different female influence. So it is more important to select the female parent in producing seeds by natural crossing (Table 2).

Table 3. Pod number comparison of female parent alternated with male parent and mixed with male parent, in arranging pattern.

Combination number	Treatment	Branch no.	Branch pod no.	Stem pod no.	Total no.
89-Ib	Alternated	3.3	8.4	8.0	16.4
89-IIb	Alternated	3.5	10.4	9.1	19.5
89-IIIb	Alternated	4.4	12.3	7.5	19.8
89-IVb	Alternated	3.5	8.8	8.9	17.7
AVERAGE		3.7	10.0	8.4	18.4

89-Ia	Mixed	3.4	9.1	7.2	16.3
89-IIa	Mixed	4.0	11.4	8.0	19.4
89-IIIa	Mixed	4.4	11.2	7.1	18.3
89-IVa	Mixed	3.9	10.7	6.7	17.4
AVERAGE		3.9	10.6	7.3	17.9

b) Effect of arranging patterns of parents in natural crossing. Statistics in Table 3 indicate that some difference in pattern of female parent alternated with male parent and mixed with male parent, the preceding pattern is better than the latter. Total number of seeds were 18.4 and 17.9, respectively. Stem pod numbers of the former was 15.1% more than that of the latter, and branch pod numbers of the former was 6.0% less than that of the latter. So it would be better to arrange female parent alternating with the male parent, in order to know which is female or male parent in field of seed, and be convenient to manage and investigate.

Conclusion: (1) A large number of seeds from natural crossing can be obtained by using male-sterile soybeans, to avoid artificial crossing work in the field. Soybean recurrent selection will be made easier.

(2) In the condition of 2:1 proportion of male to female, with male steriles, there is no difference between arrangement of parents between alternating or mixing. It is advisable to pull out fertile plants in female rows that male parent is alternated with female. The mistake of harvesting seed from male plant will be avoided, assuring seed quality.

(3) With either male or female, their crossing affinity has more important effect in producing seeds from natural crossing, so besides considering the rules of normal breeding, we must pay attention to crossing affinity, if large numbers of crossed seeds are to be produced.

(4) In terms of theory, mass male parents are more favorable than single parent, to enrich genetic foundation of crossing generations, using sterile parents. We followed this route in past years, and we are going to report results in the future. Single male parent is better if the effect of a few genes is to be elaborated by the male-sterile parent.

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He Zhihong

Zhang Junzheng

3) Genetic improvement and eco-geographical distribution of composition of soybean varieties released in Heilongjiang Province of China.

Protein and oil are the important content of soybean seeds. More than 140 soybean varieties have been released in Heilongjiang Province since 1949. It has greatly enhanced soybean production potential. However, has its chemical composition been improved? How about the relationship between chemical composition and ecological conditions of breeding regions? These questions were the focus of this study.

Materials and Methods: The 148 soybean varieties that have been developed in Heilongjiang Province since 1949 were planted with systematic groups in experimental fields of the Soybean Research Institute, Heilongjiang Academy of Agricultural Science. The first factor was the Breeding Unit, maturity period. Plot area was 2.0, 6.0 m², respectively, in 1988 and 1989. The second factor with random arrangement was varieties, in two years. They were planted in three replications, 10.0 cm seed spacing, and 66.7 cm row spacing. Main characters were investigated in the growing period; each plot was harvested at maturity; air-dry seed weight was recorded from five plants randomly selected from each plot. Content of seed protein, oil, ash and carbohydrate was analysed with a Near Infraca Analyzer. Statistical analysis was completed with an IBM computer, by MSTAT program.

Results and Analysis: a) Genetic improvement of chemical content of soybean varieties released in Heilongjiang Province. The 148 soybean varieties were divided into early, middle, and late maturing groups, according to their growing days in Harbin. The varieties of the early group (I) required less than 105 days to mature, the middle group (II) 106-120 days, and the late group (III) more than 120 days. The chemical composition of each group is shown in Table 1.

Analytic determination of chemical composition content of 12 soybean varieties released in the 50's indicated average protein content was 43.3%, oil content 19.5%, carbohydrate 32.4%, and ash 4.9%. When the chemical content of soybean varieties released in the 50's was recorded as the base, the protein content of varieties released in Heilongjiang Province had declined decade by decade over the past 40 years. There was a little increase

in oil and carbohydrate content, and some increase in ash content.

Protein content of released varieties lessened linearly from the 50's to the 70's; that content in the 80's was 1.4 percentage points less than in the 50's. Changes of protein content were different in different groups. It was reduced by 3.2 percentage points from the 50's to the 80's for Group I, and

Table 1. Chemical composition of soybean varieties released in Heilongjiang.

Days to maturity	Decade	Variety no.	Yield	Protein (%)	Oil (%)	Carbohydrate (%)	Ash (%)
I (<105)	50's	2	226.7	43.2	20.2	32.2	5.1
	80's	3	246.6	41.3	21.5	32.1	5.5
II (106-120)	50's	5	266.0	43.6	19.3	32.4	4.7
	60's	19	281.4	42.1	19.9	32.4	5.2
	70's	17	289.5	41.5	19.8	33.4	5.3
	80's	20	292.1	42.0	20.1	32.6	5.2
III (>121)	50's	5	312.2	43.0	19.4	32.6	5.1
	60's	11	302.0	41.3	19.5	33.4	5.4
	70's	17	265.7	41.4	19.9	33.7	5.4
	80's	14	335.7	41.9	19.5	33.5	5.2
Average	50's	12	278.7	43.3	19.5	32.5	4.9
	60's	30	288.9	41.8	19.9	33.0	5.3
	70's	34	277.6	41.3	19.9	33.6	5.3
	80's	37	304.9	41.9	20.0	32.9	5.2

only 1.1 percentage point for Group III. Oil content in the 60's increased by 0.5 percentage points over the 50's, but that of the 80's was the same level as that of the 50's. Oil content of Group I increased 1.3 percentage points and Group II increased 0.8 percentage points from the 50's to the 80's. Group III increased a little in the 60's and 70's, and the content of oil in the 80's returned to that of the 50's. There was a little change in carbohydrate content in Groups I and II. The content of oil in the 80's was 0.9 percentage points higher than in the 50's for Group III. Ash content of the 80's increased much more than the 50's for Groups I and II.

b) Eco-geographical distribution of chemical content of soybean

Table 2. Chemical composition distribution of varieties released in Heilongjiang.

Year	Variety no.	Protein	Oil	Carbohydrate	Ash
Songha Region					
1988	46	42.44	19.88	32.48	5.20
1989	46	41.20	19.72	33.76	5.31
average		41.80	19.80	33.11	5.25
CV%		5.12	4.53	4.40	8.87
range		37.5-44.8	17.8-21.4	31.4-36.5	4.2-6.3
Ke-Bai Region					
1988	25	42.75	20.20	31.80	5.20
1989	25	43.30	19.47	32.37	4.85
average		43.03	19.83	32.09	5.06
CV%		3.70	4.55	2.74	6.24
range		41.2-47.2	18.6-21.0	30.5-33.4	4.2-5.5
Hejiang Region					
1988	26	42.75	20.20	31.85	5.20
1989	26	43.40	19.47	32.09	5.06
average		43.03	19.83	32.09	6.24
CV%		4.14	3.49	2.97	7.45
range		39.6-44.2	18.4-20.8	32.4-35.7	4.5-5.9
Heihe Region					
1988	10	42.23	21.22	32.12	5.43
1989	10	41.22	20.19	32.50	5.09
average		41.73	20.73	32.30	5.27
CV%		6.48	5.13	3.12	5.54
range		40.9-43.8	18.9-20.8	31.5-33.2	4.8-5.4
Mudangjiang Region					
1988	9	42.40	19.68	33.49	5.40
1989	9	40.62	19.86	34.13	5.39
average		40.99	19.77	33.83	5.39
CV%		4.46	3.66	2.69	7.30
range		39.5-41.4	19.2-20.7	33.8-34.3	5.2-5.7
Nenjiang Region					
1988	20	41.68	20.55	32.27	5.50
1989	20	41.82	19.95	33.05	5.18
average		41.75	20.25	33.66	5.34
CV%		4.22	4.15	3.12	7.12
range		38.9-45.2	18.6-21.1	31.0-34.9	4.5-5.9
Suihua Region					
1988	6	41.25	19.93	33.38	5.23
1989	6	42.68	19.45	34.32	5.22
average		42.07	19.69	33.85	5.23
CV%		5.40	2.35	3.25	2.58
range		39.1-43.0	19.2-20.2	32.6-35.5	5.1-5.4

varieties released in Heilongjiang Province. Heilongjiang Province strides over 10 latitudes and 12 longitudes. There are greatly differing ecological conditions in the different regions. Songha region, the southernmost part of the province, has highest temperatures, with good soil fertility; Ke-Bai region, in the west, is a hilly region; Hejiang region, to the northeast, is a big plains region with low lying land and much more soil water; Heihe region, in the northeast part of the province, has a short growing season of about 100 days and the lowest effective cumulative temperature; Mudang-jiang region in the south part is semi-mountainous; Nenjiang region, located in the southwest, has poor soil and dry land area; Suihua region located in the mid-south has good black soil and enough cumulative temperature.

Soybean varieties with some genetic improvement of chemical content developed in different ecological condition have the characters of eco-geographical distribution (Table 2). The average protein content in different eco-geographical regions was 40.99-43.03%, and the range of protein content was 1.9 percentage points for the region with the least difference, and 7.3 percentage points for the region with the biggest range. The average of oil content in different eco-geographical regions was 19.69-20.73% and the range of oil content was 1.0 and 3.6, and 5.0 percentage points for the least and the biggest different regions, respectively. For carbohydrates it was 32.07-33.85%. For ash it was 5.06 and 5.39 percentage points.

In 85% of the regions, the coefficient of variability of ash content of varieties released was more than 5.0%. This meant that the difference between varieties was larger. The average coefficient of variability of carbohydrates was 4.4% for the biggest value, and was lower than 3.0% in general. This dictated that the difference of carbohydrate content in different eco-geographical regions was smaller. The value of protein difference and oil difference among varieties was between that of ash and carbohydrate content. The C.V.% of protein content was 3.70-6.48% and the C.V.% of oil content was 2.35-3.15%.

Released soybean varieties developed in the above mentioned regions were planted in Harbin, located in the Songha region, for two years. The results showed that protein content of soybean varieties developed in Ke-Bai region was the highest (43.03%), oil content was at the middle level (19.83%). Protein content of varieties developed in the Suihua region was 42.07%, oil content was the lowest (19.60%). Oil content of Heihe and Nenjiang regions

varieties was highest (20.73%), protein content was mid level. Protein and oil content of varieties released in Hejiang region were all low. The content was 41.55% and 19.70%, respectively. For varieties in Songha regions, protein and oil content were all low. For Mudang-jiang region varieties, protein content was the lowest (40.99%), oil content was 19.77%. Ecological conditions of Heihe and Nenjiang regions was favorable for formation of oil, because oil content of varieties developed there were all higher. So there were high oil content regions in Heilongjiang Province. The same way, high protein content varieties were easy to develop in Ke-Bai region. Suihua and Ke-Bai regions belong to a couple high regions. Hejiang region is an important soybean production region, but protein and oil content are low, so it is important to look for some new methods to promote increase in protein and oil content in the Hejiang region.

Discussion: (1) About 150 soybean varieties have been released in the last 40 years, but improvement of chemical composition has been slow. Oil content of varieties released in the first 10 years of that period only increased by 0.4 percentage points. There wasn't any increase in the second decade, and only 0.1% increment in the third ten years. Oil content related positively to yield in general. So the oil content should increase as yield is enhanced. However, things were not always the same. So increasing oil content must include in its program the increase of soybean yield so as to meet mankind's needs of high oil content soybean varieties.

(2) Protein content of soybean varieties released in the 70's was 2 percentage points lower than that in the 50's. There was some increase in the 80's. So long as we pay attention to improvement of protein content, the effect may be gotten in not too long a time.

(3) The range of protein content between different regions was 2 percentage points, the same as that of the decade, larger than that, there was the biggest difference in different regions in two years (1.2%). Comprehensive effect between climatic condition and geographical conditions was stronger than that of single climatic condition, among numbers of factors that formed the difference of protein content. This indicated that breeding means effectively improved protein content. Data indicated that improvement of protein content by breeding means alone is not very easy, to increase protein content for all varieties, but it is possible to increase protein content of a

particular variety. The range of oil content among regions was 1 percentage point; that among decades was 0.5 percentage point. The difference between two years in the region with the biggest difference of years was 1 percentage point. It was obvious that the climatic factor strongly affects oil content, among the numbers of factors, and is almost the same as the comprehensive effect between climatic condition and geographical condition. The range of decades is small, so improvement of oil in soybean breeding programs is difficult and slow.

(4) Soybean varieties released in different ecological regions planted in Harbin region for two years. The study may display characteristics of varieties that came from different ecological regions planted in the same area, as well as the effect that ecological conditions of breeding plots affects chemical composition of varieties, and analyzing characteristics of the main chemical composition distribution, but cannot display the change in different ecological regions for the same varieties. This study will continue.

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Zhang Junzheng

He Zhihong

NORTHEAST AGRICULTURAL COLLEGE

Department of Agronomy

Harbin, Heilongjiang 150030

People's Republic of China

1) Effect of planting date on amino acid composition of soybean seeds.

Soybean is famous for its high oil and protein content and balanced amino acid composition, so soybean chemical composition is an important characteristic. It has been studied by many scientists, but we have not seen any report about the effect of planting date on amino acid composition of soybean seeds. This experiment was designed to investigate this problem.

Materials and Methods: In 1986, six soybean varieties were planted in Harbin, China, on four planting dates: May 4 (I); May 11 (II); May 18 (III); May 25 (IV). The varieties were: Suinong 4, Hefeng 25, Dongnong 34, Heihe 3, Dongnong 36, and Fengshou 10. Each plot consisted of 4 rows 6 m long, with 70 cm between rows. After harvesting, the amino acid composition of the seeds was analysed by Hitachi-835 Amino Acid Analyzer.

Results and Conclusions: The amino acid content based on seed protein is relatively stable. There are no apparent differences between different planting dates and no oriented variations. The differences between varieties are slightly greater than the differences between planting dates, but they are not significant (Table 1). This indicates that the amino acid composition of soybean protein is relatively stable. Perhaps it is relevant to the important role of protein in plant metabolism.

The amino acid content based on defatted soybean meal has a greater variation, compared with those based on protein (Table 1). This is mainly due to the great variation of seed protein content. The experiment shows that protein content, on a dry matter basis, shows a great variation between varieties and planting dates. The differences between varieties are greater than those between planting dates (Table 2).

In soybean breeding, selection on amino acid content based on soybean meal is effective, but selection on amino acid content based on protein is difficult.

Table 1. Effect of planting date on amino acid composition of soybean seeds (Harbin, 1986)

	Suinong 4				Dongnong 36			
	I	II	III	IV	I	II	III	IV
ASP	11.41# 5.32^	11.57 5.74	11.51 5.78	11.43 5.48	11.65 6.10	11.59 5.66	11.63 5.37	11.61 6.24
THR	3.95 1.84	4.00 1.99	4.00 2.01	3.96 1.90	3.91 2.05	3.92 1.91	3.96 1.83	3.94 2.12
SER	5.00 2.33	5.03 2.50	5.01 2.52	5.03 2.41	5.10 2.67	5.13 2.51	5.10 2.36	5.17 2.78
GLU	20.05 9.33	20.05 9.95	20.22 10.16	20.00 9.59	20.17 10.55	19.97 9.75	19.85 9.16	20.11 10.81
GLY	4.30 2.00	4.35 2.16	4.29 2.16	4.30 2.06	4.27 2.23	4.32 2.11	4.34 2.01	4.31 2.31
ALA	4.41 2.05	4.39 2.18	4.33 2.17	4.34 2.08	4.29 2.24	4.41 2.15	4.28 1.98	4.32 2.32
VAL	5.01 2.33	5.00 2.48	4.93 2.48	4.93 2.36	4.86 2.54	4.98 2.43	4.89 2.26	4.92 2.64
ISO	4.67 2.17	4.71 2.34	4.67 2.34	4.65 2.23	4.63 2.42	4.71 2.30	4.65 2.15	4.66 2.51
LEU	7.78 3.62	7.83 3.89	7.84 3.94	7.78 3.73	7.75 4.05	7.79 3.80	7.73 3.57	7.76 4.17
TYR	3.17 1.47	3.24 1.61	3.25 1.63	3.28 1.57	3.22 1.68	3.14 1.53	3.22 1.49	3.19 1.72
PHE	5.06 2.35	5.25 2.61	5.18 2.60	5.12 2.46	5.22 2.73	5.14 2.51	5.26 2.43	5.23 2.81
LYS	6.39 2.97	6.31 3.13	6.23 3.13	6.40 3.07	6.27 3.28	6.36 3.10	6.30 2.91	6.32 3.39
HIS	2.51 1.17	2.55 1.27	2.61 1.31	2.61 1.25	2.57 1.34	2.50 1.22	2.57 1.19	2.59 1.39
ARG	7.87 3.66	7.66 3.80	7.87 3.95	7.82 3.75	7.77 4.07	7.45 3.63	7.40 3.42	7.57 4.07
PRO	4.00 1.86	4.11 2.04	4.03 2.03	4.04 1.94	4.17 2.18	4.15 2.02	4.36 2.01	4.19 2.25
TRY	1.47 .68	1.26 .63	1.30 .65	1.39 .67	1.28 .67	1.40 .68	1.47 .68	1.31 .70
CYS	1.66 .77	1.55 .77	1.56 .78	1.68 .81	1.71 .89	1.81 .88	1.76 .81	1.68 .90
MET	1.27 .59	1.14 .56	1.18 .59	1.25 .60	1.18 .62	1.22 .60	1.23 .57	1.12 .60

= % Amino acid content based on protein.

^ = g/100g (amino acid gram/100 gram defatted soybean meal) Amino acid content based on defatted soybean meal.

I = 4 May; II = 11 May; III = 18 May; IV = 25 May.

Table 2. Effect of planting date on protein content (Harbin, 1986)

	Suinong 4	Dongnong 36
I	43.67#	45.10
II	42.41	44.14
III	42.97	43.22
IV	43.11	44.86

= % On dry matter basis.

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Zhang Guodong

Wang Jinling

1) Accumulating pattern of protein and fat during developing seed in three soybean types.

Protein and fat content of the mature seed in soybean depend on their accumulation during development. Wolfe et al. (1942) found that protein and fat of the mature seed were probably largely from substances brought into the seed from other parts of the plant, at the time of synthesis, rather than the carbohydrates already present in the seed. Rubel et al. (1972) determined the protein percentage of the seed and found that percentage protein remained approximately constant through podfilling. On the other hand, Blis and Howell (1963) indicated that protein percentage increased from 26% to 47% during podfilling. Sale and Campbell (1980) also observed that protein accumulation occurred continuously until maturity. There are differences among the results

mentioned above. In order to obtain systematically accumulating characteristics of the protein and fat in developing seeds, three types of five varieties were used in this research.

Materials and Methods: Three types of five varieties, which were similar in maturity period and different in protein and fat content, were grown in the field on the Xiang Fang Experimental Farm at Harbin in 1987. They included (a) the high-protein types, Dongnong 33 and Har 82-5775; (b) the high-fat types Dongnong 38 and Heinong 31; and (c) the type high in both protein and fat, Dongnong 86-601. Individual pods were tagged on each variety at R5 stage and expressed as days after flowering (DAF). The pods tagged were harvested at intervals of five days for analysis. The sample seeds were placed in an oven for drying, after recording the numbers and fresh weight. Then all dried samples were weighed and the percentage of moisture calculated before storing them in a dessicator until they were analyzed for protein and fat. The protein was measured by using the Kjeldahl procedure (factor 6.25) and fat by the residue method.

Results and Discussion: The development of soybean seeds in five varieties from 36 to 76 days after flowering were observed in this study. Accumulating trends between protein and fat, expressed in terms of milligrams per seed, were basically similar among the three types (see Fig. 1) and they increased continuously in amount until maturity in either the high-protein type and both high in protein and fat type or high-fat types. But there was a slight decrease in amount of protein and fat per seed in the high-fat type during the last week of senescence, which was similar to the result observed by Hill and Breidenbach (1974) and Sale and Campbell (1980).

The result that Dongnong 33, of the high-protein type, had the highest fat milligrams per seed, was caused by its larger seed weight, suggesting that when the changes of protein and fat were expressed in milligrams per seed during seed development, they were affected by seed size.

At 36 DAF, the amounts of protein and fat in the five varieties represented 6-14% and 3-5% of the total protein and fat in mature seed (mg/seed) respectively. This indicated that more than 86% of the protein and 95% of the fat were mainly accumulated after 36 DAF.

The content of protein and fat on a fresh weight basis kept rising

during seed development in all three types. From 36 to 66 DAF, they rose steadily, and thereafter increased rapidly until maturity. The results showed that the accumulation of the protein and fat in this period might be due to the moisture-free state of the mature seed. However, protein content on a fresh weight basis for high-fat types was higher than that for high-protein types at 71 DAF. It could be moisture-free sooner in the prior type than in the latter. This result showed that the greater amount of protein that the seed contained, the slower the loss of its water, because protein had the characteristics of being moisture loving. (See Fig. 2.)

Both protein and fat content expressed on a dry weight basis could reflect their real levels in mature seed rather than those either expressed as milligrams per seed or on a fresh weight basis. In contrast to the obvious increase of protein and fat on a fresh weight basis, the percentage of protein on a dry weight basis tended to be relatively constant during podfilling, from 36 to 71 DAF, and fat content showed the same trend after 46 DAF. These results also showed that the accumulation of fat was later than that of protein, and this conflicted with the results obtained by Blis et al. (1963). However, there are differences for the change of protein content on a dry weight basis during developing seed in the three types of five varieties. In the high-protein type, the protein content of Dongnong 33 tended to increase steadily, which was in agreement with the results obtained by Sale et al. (1980). And that of Har 82-5775 declined slightly from 36 to 51 DAF and thereafter increased rapidly (data not shown). But in the high-fat type, content of protein tended to not only not increase but also to decrease during maturation. Then in both high types, the trend of protein content for Dongnong 86-601 was somewhat similar to the high-protein type.

In comparison to protein content, the change in fat content on a dry weight basis was preliminarily divided into three periods, according to their appearance at different times. In the first period from 36 to 46 DAF, the fat content increased rapidly and had a similar trend among the three types. For the second period, from 46 to 61 DAF, the fat content remained relatively unchanged for the high-protein type, but dropped down before rising up for high-fat types and both high types. The third period, from 61 to 71 DAF, was an important period, in which was decided the fat content in mature seed of each variety. In this period, the fat content in high-fat and both high types increased continuously, but decreased in the high-protein type. The decrease

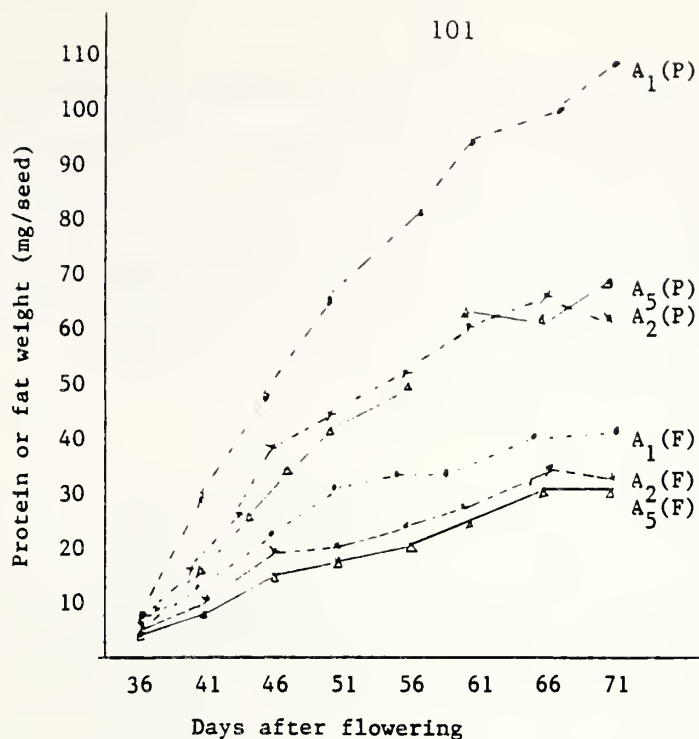


Fig. 1. Change in protein or fat weight (mg/seed) in developing seeds of three soybean types.

---•--- A₁ DN33 -x-x-x- A₂ DN38 ▲—▲— A₅ DN86-601

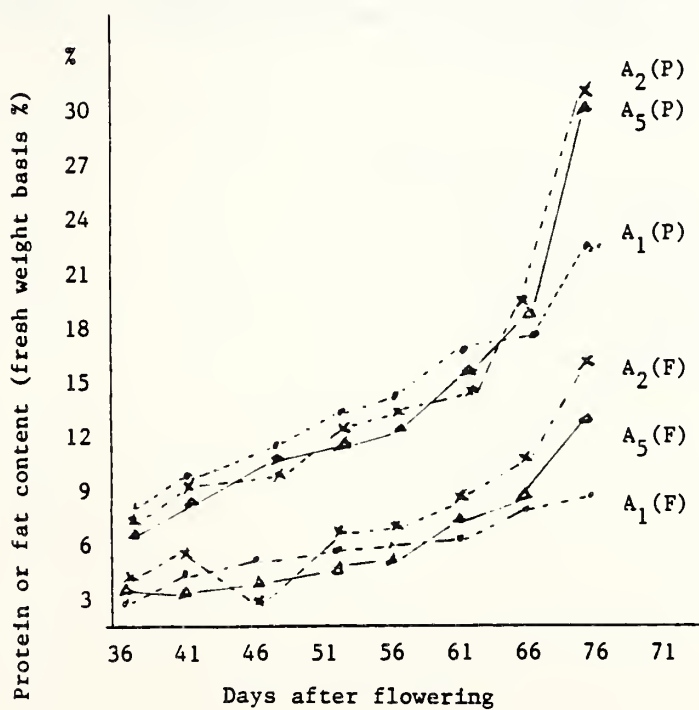


Fig. 2. Change of protein or fat content (fresh weight basis %) in developing seeds of three soybean types.

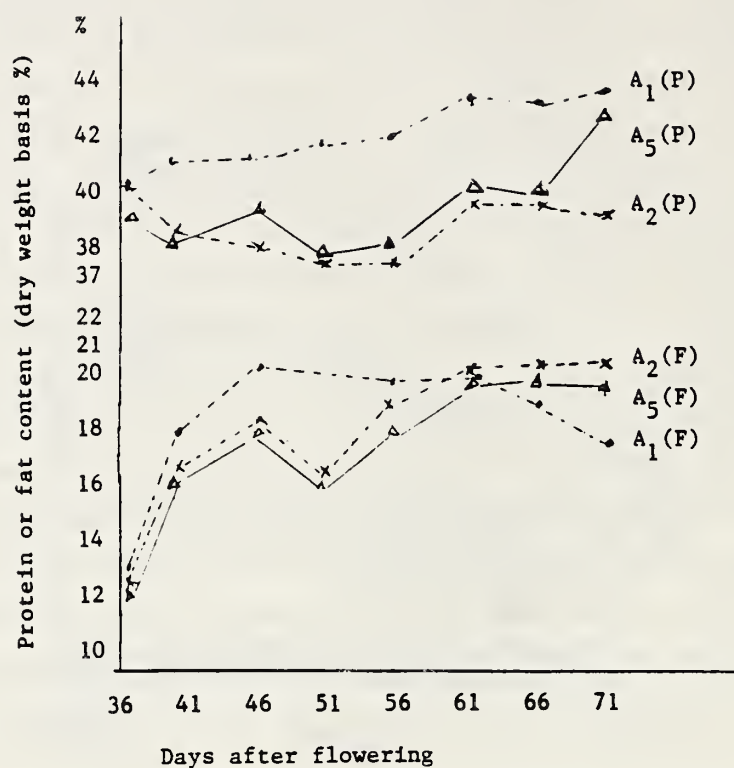


Fig. 3. Change of protein or fat content (dry weight basis %) in developing seed of three soybean types.

in fat for high-protein types might be related to photosynthetic assimilate introducing which declined during senescence (Schulzer et al., 1976) and the loss in fat might well have provided additional carbon for protein synthesis (Sale et al., 1980). The results above indicated that there exists a final increase or decrease in the mature seed for both protein and fat content, which express the properties of each variety of soybean.

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Qiu Lijuan

Present address:

Institute of Crop Germplasm
Resources of the Chinese
Academy of Agricultural
Sciences. 30 Bai Shi Qiao
Road, Beijing 100081, PRC.

Wang Jinling

Meng Qingxi

SOYBEAN RESEARCH INSTITUTE
 NANJING AGRICULTURAL UNIVERSITY
 Nanjing, Jiangsu 210014
 People's Republic of China

1) Inheritance of resistance to the beanfly (*Melanagromyza sojae* Zehntner) in soybean.

Ten possible crosses plus two reciprocal ones among five parents, two highly resistant (HR), two highly susceptible (HS), and one moderate (M) (Table 1), were used to study the inheritance of resistance of soybeans to the beanfly (*Melanagromyza sojae* Zehntner).

Table 1. Parents and their resistance rank in soybean.

Parent	Code	NIS*	Resistance rank
N3498	A	0.73	HR
N2980	B	0.79	HR
N10291	C	4.25	HS
N3688	D	3.73	HS
N5460	E	1.68	M

*NIS = Number of insects in stem.

The number of insects in the stem (NIS) was used as the indicator of resistance and the standard--variety grade system (Gai, 1990) was applied to grade the response of resistance.

The results from six crosses between HR and HS (Tables 2, 3) indicated that the inheritance of resistance was controlled by a single gene. The HR parent has a dominant gene (Rms), while the HS parent has a recessive gene (rms). The results from two reciprocal crosses indicated that no cytoplasmic effect was observed.

It seemed that the expression of resistance was modified by environmental conditions, rather than by polygenes.

In addition, there was no genetic variation observed in the F₂ or F₃ for

Table 2. The segregation of crosses between HR and HS in soybean.

Cross	Generation	R	S	Total	Segregation ratio tested	X ²	P
A x B	F ₂	209	85	294	3:1	2.19	0.10-0.25
	F ₃	60	26	86	3:1	0.99	0.25-0.50
	B ₁ F ₂	580	94	674	7:1	1.16	0.25-0.50
B x A	F ₂	183	71	254	3:1	1.03	0.25-0.50
	F ₃	70	25	95	3:1	0.01	0.90-0.95
B x C	F ₂	172	67	239	3:1	1.01	0.25-0.50
	F ₃	64	30	94	3:1	2.04	0.10-0.25
A x D	F ₂	190	77	267	3:1	1.90	0.10-0.25
	F ₃	70	29	99	3:1	0.76	0.25-0.50
C x D	F ₂	196	73	269	3:1	0.55	0.25-0.50
D x C	F ₂	200	71	271	3:1	0.15	0.50-0.75

Table 3. Testing the homogeneity of the segregation ratio (3:1) in crosses between HR and HS, in soybean.

Gener- ation	No. of crosses	Source of variation	R	S	Total	X ²	df	P
F ₂ plant	6	Pooled	1150	444	1594	6.93	1	
		heterogeneity				1.04	5	0.90-0.95
		Total				7.97	6	0.10-0.25
F ₃ family	4	Pooled	264	110	374	3.88	1	
		heterogeneity				0.88	3	0.75-0.90
		Total				4.72	4	0.25-0.50

the crosses of HR x HR and HS x HS, which indicated existence of allelism of the resistant genes in the HR parents, as well as in HS parents.

The results from four crosses between HR and M, and between HS and M seemed to be somewhat different from those between HR and HS, and might be a challenge to the above conclusions. Thus, further study is needed.

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Gai Junyi

Liu Fanyi

Cui Zhanglin

Xia Jikang

Ma Yuhua

SOYBEAN INSTITUTE
JILIN ACADEMY OF AGRICULTURAL SCIENCES
Gongzhuling, 136100
Jilin Province
The People's Republic of China

1) Superoxide dismutase zymogram patterns and their geographical distributions of semiwild soybean (*G. gracilis*) in the Northeast of China.

Introduction: Many previous studies have shown that superoxide dismutase (SOD) is able to prevent organisms from being injured and has defense against aging, since it can catalyze the dismutation of the superoxide negative ions free radical to molecular oxygen and hydrogen peroxide. Therefore, SOD has a bearing on resistance or tolerance to heat, drought, salt, SO₂, O₃, and cold (Beauchamp et al., 1973; Dhindsa et al., 1981; Kalir et al., 1981; Lee et al., 1982; Stewart et al., 1980, and Tanaka et al., 1980).

There were also many investigations carried out on soybean SOD in recent years. These studies were concerned with the SOD patterns and their distribution in wild and cultivated soybeans in China (Xu Bao et al., 1990), the distribution of SOD in the soybean plant cell (Lee et al., 1982), the change of SOD during seed germination and development (Zhuang et al., 1990 and 1991), and the heredity of SOD. But there were no reports of the SOD of semiwild soybean. Knowledge of semiwild soybean biological characters is important for uses and study of original area and evolution of soybean.

Materials and Methods: One hundred twenty three semiwild soybean genotypes from the Northeast of China were used in this experiment. All of these samples were provided by The Soybean Institute of the Jilin Academy of Agricultural Sciences. Gel electrophoresis of SOD was made according to the method described by Luo Guanghua et al. (1985)

Results: 1) SOD patterns: The results in Table 1 and Figure 1 showed that the bands of SOD isoenzyme of semiwild soybean in the Northeast of China

*--This project supported by NNSF of China.

is the same as that of the wild and cultivated soybean, and have nine bands called SODa, SODblb2b3, and SODclc2c3c4c5, respectively. There were five patterns of SOD's displayed in the semiwild soybean in the Northeast of China: they are types I, II, III, IV, and VII. The pattern VII was only found in the semiwild soybean at present.

Table 1. SOD patterns and their distribution in semiwild soybeans in the Northeast of China.

Province	No. of sample	SOD patterns and gene frequency(%)				
		I	II	III	IV	VII
Heilongjiang	21	0	100.00	0	0	0
Jilin	21	33.33	66.67	0	0	0
Liaoning	81	19.75	69.14	6.17	3.70	1.23
Total	123	18.70	73.98	4.07	2.43	0.81

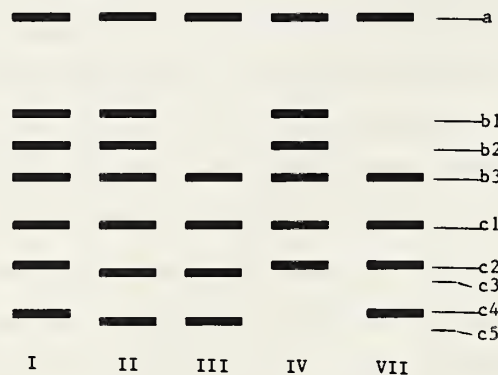


Figure 1. SOD zymogram patterns in semiwild soybeans in Northeast China.

2) Gene frequency and geographical distribution of SOD: It can be seen from Table 1 that the SOD pattern of most of the samples belonged to type II (73.98%) and I (18.70%). Only a few of the samples belonged to other types. The SOD pattern of semiwild soybeans was richer as latitude decreased. For example, there was only one kind of SOD pattern in the semiwild soybeans of Heilongjiang Province; there were two kinds in the samples from Jilin

Province; and there were five kinds of SOD patterns in the samples from Liaoning Province.

3) Relationship of SOD patterns and some characteristics of the seed: The results shown in Tables 2 and 3 show that there was no direct relationship between SOD pattern and seed coat color, bloom, or grain weight.

Table 2. Relationship of SOD pattern and color and bloom of seedcoat of semiwild soybean.

Zymogram	Color of seedcoat						Bloom	
	Black	Brown	Dk. green	Green	Yellow	Double color	Yes	No
I	9	7	0	2	4	1	16	7
II	44	32	4	5	6	0	61	30
III	3	2	0	0	0	0	2	3
IV	0	2	0	0	1	0	1	2
VII	0	0	0	0	1	0	0	1
TOTALS	56	43	4	7	12	1	80	43
%	45.53	34.96	3.25	5.69	9.76	0.81	65.04	34.96

Discussion: 1) Previous studies show that there were six SOD patterns found in the wild and cultivated soybeans in China (Xu Bao et al., 1990). Type VII of SOD pattern found in the semiwild soybean may be the result of natural hybridization of wild type (Type I) and cultivated type (Type III), because we have found that this type exists in the cross of wild (I) and cultivated soybeans (III). Therefore, it is supposed that there is natural hybridization of wild and cultivated soybeans. On the other hand, the pattern of SOD of most semiwild soybeans was the same as that of the cultivated and the wild soybeans, and the frequency of Type I and Type II of semiwild soybeans was once between that of wild and cultivated soybeans. It seems that, in the path of evolution of semiwild soybeans from the wild type, many small genetic mutations may have accumulated, and then evolved to the cultivated soybean; this may be the main path of evolution to the cultivated soybean. Therefore, the following two paths may have existed in the process of soybean evolution:

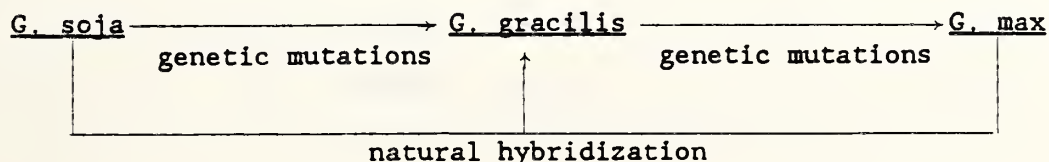


Table 3. Relationship of SOD pattern and grain weight.

Zymogram	No. of samples	Weight of 100 seeds (g)						
		3.0-4	4.01-5	5.01-6	6.01-7	7.01-8	8.01-9	9.01-10
I	23	4	9	5	2	0	0	3
II	91	34	19	15	9	5	4	5
III	5	2	1	1	1	0	0	0
IV	3	2	0	1	0	0	0	0
VII	1	1	0	0	0	0	0	0
Total	123	43	29	22	12	5	4	8
%		34.96	23.58	17.89	9.26	4.07	3.25	6.50

2) Our study showed that the response to photoperiod and day and night temperature, protein content of seeds and gene frequency of *Ti* of wild and cultivated soybeans from 35-40°N region in China, especially the Yellow River region, was most close, so we supposed that this region may be the original area of cultivated soybean (Xu Bao et al., 1986). This study showed that the SOD pattern of semiwild soybeans of Liaoning Province (about 43-39°N) was richer than that of both Heilongjiang (north of 44°N) and Jilin (about 41-46°N) Provinces. This may be because the Liaoning Province is located in or near the area of origin of soybeans.

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Zhuang Bingchang

Xu Bao

2) Genotypes of SBTi-A₂ and Spl of cultivated soybean in Northeast China.

There have been many investigations carried out on the SBTi-A₂ and Spl seed storage proteins in soybean seeds (Hymowitz et al., 1979, 1981; Xu Bao et al., 1983; Wang Yantong et al., 1986). This is the first report on SBTi-A₂ and Spl of all the cultivars in Northeast China.

Materials and Methods: All of the samples used in this study were from the germplasm laboratory of our institute. Extraction of seed storage protein followed the method described by Hymowitz et al. (1972). Electrophoresis followed the method described by Hu Zhiang et al. (1983). SBTi-A₂ markers were from Sigma Co.

Results and Discussion: It can be seen from the data in Table 1 that most of the cultivars (99.56%) from Northeast China belonged to Ti^a, similar to results from many cultivars from all of China (Wang Yantong et al., 1986). But the genotype and frequency of Ti was different among different provinces. In the southern part (Liaoning Province), most of the cultivars were Ti^a; only one genotype, called 'Gaoli Huang' from Dandong City, was Ti^c. This cultivar may have been introduced from Korea, based on the cultivar name and cultivar area. Therefore, all of the cultivars from the southern part were Ti^a. This is similar to results from Wang Yantong (1986). There were two kinds of Ti genotypes in the samples from Jilin Province. Most of these samples (99.75%) were Ti^a. Two genotypes, from the 44°N region, were Ti^b. There were three kinds of Ti genotypes in the northern part (Heilongjiang Province), Ti^a genotype was 99.03%, six samples were Ti^b, and these six cultivars were from a wide area. One sample, from the 45°N region, was Ti^c.

Table 1. Genotypes and gene frequency of SBTi-A₂.

Region (Province)	No. of samples	Ti ^a		Ti ^b		Ti ^c		titi
		No.	%	No.	%	No.	%	
South (Liaoning)	744	743	99.87	0	0	1	0.13	0
Central (Jilin)	814	812	99.75	2	0.25	0	0	0
North (Heilongjiang)	719	712	99.03	6	0.83	1	0.14	0
Totals	2277	2267	99.56	8	0.33	2	0.09	0

There were four kinds of Spl genotypes in the cultivars of northern and central parts of northeast China. They were Spl^a, Spl^b, Spl^{an}, and spl. Only two kinds of Spl genotypes (Spl^a and Spl^b) existed in the samples from the southern part. The genotypes Spl^a and Spl^b were distributed in all of these three provinces. Spl^{an} was only distributed throughout the central and northern parts, and most of this genotype came from the plains area. Most of the genotype spl came from the mountain area of the eastern part.

Table 2. Genotypes and gene frequency of Spl.

Region	No. of samples	Spl ^a		Spl ^b		Spl ^{an}		spl	
		No.	%	No.	%	No.	%	No.	%
South	744	37	5.0	707	95.0	0	0	0	0
Central	814	47	5.8	754	92.6	8	1.0	5	0.6
North	719	20	2.8	686	95.4	5	0.7	8	1.1
North	719	20	2.8	686	95.4	5	0.7	8	1.1
TOTAL	2277	104	4.6	2174	94.3	13	0.6	13	0.6

The preceding results showed that there were different genotypes of Ti and Spl in the soybean cultivars of northeast China. These results were similar to some other regions based on our results for the the analysis of the cultivars in China (not published). The genotypes of Ti and Spl were more complex as the latitude increased in northeast China. In addition, we found that the percentage of Ti^a and Spl^b of cultivated soybean was higher than that of semiwild ones, and the latter was higher than that of wild soybean in China. It seems that the frequency of Ti^a and Spl^b was correlated with the evolutionary level. We also found that the frequency of Ti^a and Spl^b was higher as the cultivated history increased. This seems that the frequency of Ti^a and Spl^b may have some correlation with some economic, resistance, and adaptation characters.

Zhao Shuwen

Zhou Shuhua

Hu Mingxiang

Meng Xiangxun

Li Aiping

Wang Shuming

3) Protein and oil content of soybean seed as influenced by years and location.

Previous studies (Hu et al., 1990; Carter and Hooper, 1942; Taira and Taira, 1971) indicated that protein and oil content of soybean seed were not only conditioned by their inheritance, but also influenced by the environmental conditions where the crop was grown. That is, soybean seed protein and oil content vary among varieties and the same variety planted in various years or various locations also showed differences. The objective of this investigation is to explore what amount of protein and oil variation are due to years and locations or to give a generalization about the range of variation.

Materials and Methods: Four soybean varieties (Jilin No. 20, Jilin No. 28, Tongnon No. 9, and Jiujiao 7714), differing in protein and oil content, were grown at five Jilin locations (Jiuzhan, Huadian, Yushu, Changchun and Tonghua) at latitudes of from 42° 32' to 44° 21'N and longitudes of from 125° 18' to 126° 45' E, from 1987 to 1989. Seed samples of each of the four varieties from each location were collected and determined for protein and oil content by Model 51A.

The effects of variety, year, location, and year x location interaction on the percentage of protein, percentage of oil, and the sum of protein and oil were estimated with analysis of variance. The significance of the difference among the means calculated by variety, year and location was tested with LSR.

Results and Discussion: An analysis of variance of the data indicated that the effect of variety, year, location and year x location interaction on protein and oil percentages and the sum of protein and oil content were significant or highly significant (Table 1), suggesting that the protein, oil and protein + oil percentages could vary markedly with the crop years and locations. This was consistent with previous reports (Hu et al., 1990; Carter and Hooper, 1941, and Taira and Taira, 1971). It was expected that significant varietal differences occur, since they were selected on the basis of protein and oil content. Furthermore, all the variance components due to the location for the protein, oil, and protein + oil were consistently larger than those due to the year.

The mean, standard deviation, and variation coefficient of each of the

four varieties for protein, oil, and protein + oil are presented in Table 2. Standard deviations and variation coefficients representing the coordinated effect of year with location were not fairly varied with the varieties although their mean difference existed significantly, indicating variability among varieties for the protein, oil, and protein + oil content was similar, no matter how high or low the varieties are in protein and oil.

On the average across both years and locations, it was observed that the percentage of protein varied with a range of $\bar{X} \pm 1.587\%$, percent oil with a range of $\bar{X} \pm 0.707\%$, and sum of protein and oil with a range of $\bar{X} \pm 1.156\%$ at 68% probability (Table 2).

The means, separately calculated by year or by location of the four varieties, are given in Table 3. Among the five locations, Tonghua gave the highest protein; protein + oil was higher than Jiuzhan and Huadian. The latter two were higher than Changchun and Yushu. For the mean oil, Tonghua was the lowest among the five sites, and Changchun and Yushu were lower than Jiuzhan and Huadian. In addition, the means of protein, oil, and protein + oil percentages were significantly different from year to year.

Standard deviations and various coefficients after location's means represented the variability due to the years, and those after year's means represented that due to the locations (Table 3). It was shown that the variation due to location was larger than that due to year. The averaged variability for locations for protein was $\bar{X} \pm 1.423\%$, for oil $\bar{X} \pm 0.585\%$ and for protein + oil $\bar{X} \pm 1.100\%$ at 68% probability. The variability from the crop years for protein, oil, and protein + oil were $\bar{X} \pm 1.017\%$, $\bar{X} \pm 0.602\%$, and $\bar{X} \pm 0.944\%$, respectively.

It was concluded that it could be more precise to have protein and oil content of a soybean evaluated or determined with more samples of seed from at least two or three years or locations.

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Table 1. An analysis of variance of data.

Source of variation	DF	SS	MS	F-value	F 0.05	F 0.01
----- Protein percentage -----						
Varieties	3	181.973	60.657	409.071	2.83	4.29
Years	2	2.053	1.026	6.919	3.22	5.55
Locations	4	41.233	10.308	69.517	2.59	3.80
Y X L	8	3.383	0.423	2.851	2.17	2.96
Error	42	6.228	0.148			
Total	59	234.870				
----- Oil percentage -----						
Varieties	3	17.614	5.871	58.830	2.83	4.29
Years	2	3.028	1.541	15.439	3.22	5.55
Locations	4	2.503	0.626	6.271	2.59	3.80
Y X L	8	3.069	0.384	3.844	2.17	2.96
Error	42	4.192	0.099			
Total	59	30.495				
----- Protein + Oil percentage -----						
Varieties	3	45.776	15.295	103.182	2.83	4.29
Years	2	9.350	4.675	31.614	3.22	5.55
Locations	4	24.153	6.038	40.832	2.59	3.80
Y X L	8	4.162	0.528	3.518	2.17	2.96
Error	42	6.211	0.148			
Total	59	89.652				

Table 2. Mean protein, oil, and protein plus oil of each of the four soybeans and their variations due to both crop years and location.

Variety	Mean*	Range	Sx	C.V. %
----- Protein percentage -----				
Jilin No. 20	40.23 c	47.46 - 41.51	1.365	3.392
Jilin No. 28	45.81 a	43.34 - 49.48	1.634	3.567
Tongnon No. 9	44.13 b	41.56 - 46.90	1.484	3.364
Jiujiao 7714	39.96 d	37.42 - 43.56	1.864	4.665
Mean	42.54		1.587	3.747
----- Oil percentage -----				
Jilin No. 20	19.64 b	18.82 - 21.28	0.627	3.115
Jilin No. 28	17.66 d	15.71 - 18.51	8.577	3.270
Tongnon No. 9	18.80 c	17.72 - 19.51	0.701	3.729
Jiujiao 7714	21.89 a	19.30 - 22.96	0.938	4.219
Mean	19.50		0.707	3.583
----- Protein + Oil percentage -----				
Jilin No. 20	60.18 d	58.40 - 61.63	1.216	2.021
Jilin No. 28	63.48 a	61.00 - 65.93	1.308	2.061
Tongnon No. 9	62.96 b	61.42 - 65.97	1.236	1.963
Jiujiao 7714	61.96 c	60.80 - 63.47	0.863	1.392
Mean	62.14		1.156	1.859

*The varied letter after the mean column representing the significance of difference at 5% probability level.

Table 3. Mean protein, oil, and protein + oil of each location or each year and their variations from location or crop year.

Location or year	Protein percent			Oil percent			Protein + Oil percent		
	Mean	Sx	C.V. %	Mean	Sx	C.V. %	Mean	Sx	C.V. %
Changchun	41.90c	1.997	2.618	19.43c	0.495	2.547	61.34b	0.893	1.455
Yushu	41.83c	1.218	2.911	19.61bc	0.695	3.543	61.45b	1.615	2.628
Jiuzhan	42.13bc	0.982	2.331	19.83b	0.541	2.727	61.97b	0.503	0.812
Huadian	42.35b	0.828	1.955	19.95a	0.496	2.487	62.82a	0.582	0.926
Tonghua	44.20a	0.961	2.174	18.54d	0.781	4.213	62.62a	1.126	1.798
Mean	42.54	1.017	2.398	19.49	0.602	3.102	62.13	0.994	1.542
1987	42.07b	1.279	3.039	19.84a	0.588	2.863	62.23b	0.944	1.517
1988	43.63a	1.558	3.571	19.62b	0.562	2.964	63.25a	1.236	1.954
1989	41.91b	1.432	3.417	19.06c	0.651	3.419	61.00c	1.119	1.853
Mean	42.54	1.423	3.342	19.50	0.585	3.082	62.15	1.100	1.768

Meng Xiangxun

Wang Shuming

Li Aiping

Hu Mingxiang

CZECHOSLOVAKIA

MENDELEUM RESEARCH CENTRE OF GENETICS

University of Agriculture Brno

691 44 Lednice na Morave

Czechoslovakia

1) Mendelistic factors and heredity of quantitative traits in soybean.

Abstract: In F_2 and F_3 generations in soybean the traits plant height, number and weight of seeds were studied. The variability of these and similar traits is presently thought to be conditioned by an integrated effect of polygenous systems, where participating loci enter into various types of interaction, their impact being considerably influenced by the environment given. The article demonstrates that it is possible to model this multiple causation by means of a few independent factors. To discern among them is a matter of precise measurement.

Introduction: Since the early 20th century the contradiction between the mendelistic theory of heredity and some more recent approaches to understanding the heredity of quantitative traits has been under more or less intensive discussion (see e.g., Lee and Parsons, 1968). In the forties the term polygenetic heredity was introduced (Mather, 1949) and biometrics was dominated by purely statistical approaches. This has, admittedly, helped explain the continual variability of quantitative traits but, on the other hand, has led to a considerable reduction of genetic models. Unfortunately, soybean is an exemplary plant for the obstacles it puts in the way of using classical biometric models for analyzing hybrid populations: crossing is extremely difficult and the success rate minimal, only a few F_1 seeds can be thus obtained. But, of course, it is necessary to select and evaluate the material as soon as in F_2 and F_3 generations (Kadlec, 1988). In this contribution we are concentrating on how to establish the number of independent factors conditioning some metric traits.

Methods: Our efforts are based on the probability space (Ω, Γ, P) with 4^k elements, the field Γ representing a set of genotype classes $(G_i; i=1, 2, \dots, 3^k)$ where k is a number of factors-elements (Mendel, 1886) and P

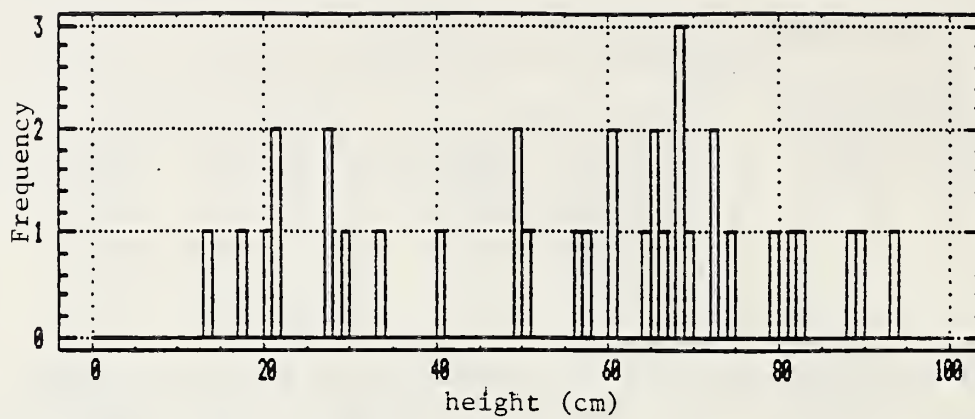


Figure 1. (a) Frequency histogram

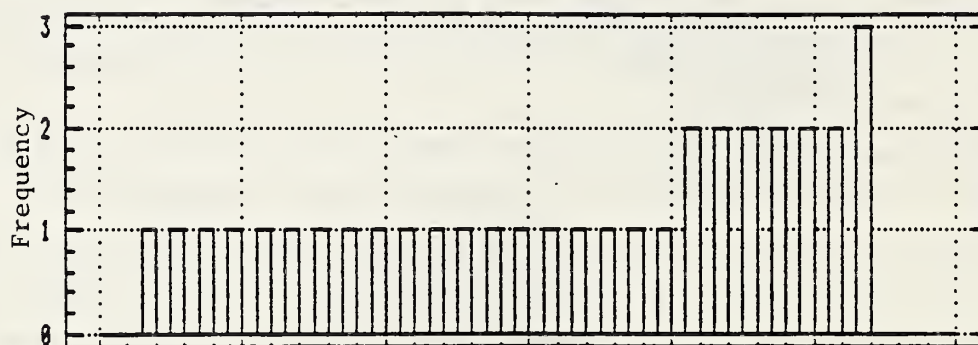


Figure 1. (b) Real data (sorted)

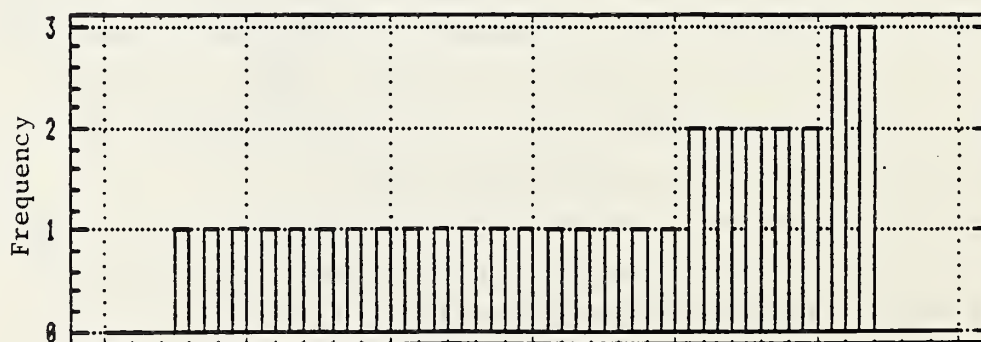


Figure 1 (c) Simulation (4 factors)

is the relative frequency of the classes G_i . This space serves as a basis for modelling probability distribution ($P(G_i)$; $i=1,2,\dots,3^k$). The method itself consists in computer simulation of random selections from the space Ω and their subsequent comparison with the real selection.

Materials and Results: Crossing of two soybean varieties Sluna and Schae-01 was carried out through the technique of castration in 1987. Flower color was chosen as the marker trait for identifying crossing success. Only one evident hybrid in F_1 was obtained and it gave 34 F_2 plants.

The distribution of these plants as to height is given in Fig. 1a, the distribution being 1 cm. The max-min range is here approximately 1 m. The maximal number of factors that can be extrapolated at the given precision and range is 4, because $1/3 \approx 0.01$ [m]. One thousand random selections were simulated from the space Ω for $k=4$. The number of genotype classes G_i was calculated as being 25.64 ± 0.06 , so that the interval of confidence for 3 can be set at [20;31]. This is in accordance with the observed number of phenotype classes. For graphic demonstration of the model distribution and their comparison with the real distribution see Fig. 1 b, c. The distributions are ordered according to frequency in individual classes. An analogical layout can be seen in the analysis of distributions for weight and seeds number.

The above calculations are based on one experiment only and need further verification. Such verification could be arrived at in two ways:

- a) through analysis of further crossings at F_2 Generation, preferably in more than one environment (each environment has to undergo a separate analysis),
- b) through analysis of the progenies of the starting population in F_2 - i.e., going over to F_3 analysis.

The difficulties involved in artificial hybridization of soybean (Kadlec, 1988) made it necessary to go the latter way, even if it is more demanding methodically. The former way, however, could be utilized in experiments done with maize (Letal, 1990). There the distribution structure of ordered frequencies of phenotype classes proved to be invariant to the environment conditions.

In 1988, 34 offspring populations were obtained from the original F_2 population. Each offspring population was subjected to a selection giving on average 30 plants. The analysis of F_3 generation was methodically identical to that of the F_2 generation. Let it be noted that the simulation experiments

showed a considerable range in number of elements in individual classes, which is understandable in view of the theoretical dividing ratio. This was projected into other figures; e.g., rather broad intervals of confidence and simulation extrapolations.

Conclusion: Almost 40 years ago, an article was published (Larroque, 1950) surveying all practical knowledge from 12 years' experimental work with different crops, especially maize. The author supported the idea about constant genetic complexes that are transferred unchanged to following generations. This idea was developed even later. Due to links between individual loci along the chromosome, these complexes are more or less identified with the whole chromosomes (Parsons, 1963; Franklin and Lewontin, 1970; Slatkin, 1972; Hanacek, 1986; Paterson *aj.*, 1988).

In accordance with this and on the basis of our method, our results allow a similar interpretation. They do not exclude the hypothesis that it is whole chromosomes that influence the variability of a trait. Establishing the amount of contributions by individual chromosomes is, however, beyond the aim of this article. The results do not contradict the biometric approach either; for rice, for example, the number of effective factor pairs influencing height was found to be 2-4 (Mohamed and Hanna, 1964).

It is shown that the mendelistic description of quantitative heredity, is, as a principle, feasible.

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J. Letal

M. Kadlec

2) Anther cultures in soybean.

Introduction: Anther cultures, in general, can expressively influence effectiveness of the breeding process. However, there are no reports until now on any success of anther culture in soybean. We decided to resolve the problem through analyzing the course of development of microsporogenesis and its phenotypic reflection in the position and size of the flower bud as well as through screening media and cultivars from the Mendeleum gene pool stock. In this sense some pieces of knowledge were drawn on that were reported by Ivers et al. (1974).

Materials and Methods: Four cycles of six-factor trials were used in the creation of media where each of the factors was presented at five levels, i.e., there were 25 different medium types (Table 1) in each of the cycles. We present results of the fourth cycle, a group of variable factors being presented with a group of basic components of the medium in Table 2. Of the cultivars the following lines were used: L-20, L-21, L-23, L-25, L-45, L-51 and L-16K. Staining with iron acetocarmine and the Nu-2 microscope (Zeiss Jena, enlargement of anthers 12.5x4 obj., enlargement of microspores 12.5x25 or 12.5x63 obj.) were applied to identify development of the microspores. Part of the plants with flower buds identified as optimum ones were placed on the MS medium and kept in cold at a temperature of $2 \pm 1^{\circ}\text{C}$ for 24 hours or longer,

being then sterilized and used. The second portion of the explants with the buds was instantly sterilized with 15% calcium hypochlorite solution for 15 min., and used after fourfold rinsing in sterile water. The anthers

Table 1. Scheme of experimental arrangement of six factors.

N	1	2	3	4	5	6
1	1	1	1	1	1	1
2	1	3	3	3	3	3
3	1	2	2	2	2	2
4	1	5	5	5	5	5
5	1	4	4	4	4	4
6	3	1	3	2	5	1
7	3	3	2	5	4	1
8	3	2	5	4	3	3
9	3	5	4	1	3	2
10	3	4	1	3	2	5
11	2	1	2	4	3	5
12	2	3	5	1	2	4
13	2	2	4	3	5	1
14	2	5	1	2	4	3
15	2	4	3	5	1	2
16	5	1	5	3	4	2
17	5	1	5	3	1	5
18	5	2	1	5	3	4
19	5	5	3	4	2	1
20	5	4	3	2	5	3
21	4	1	4	5	2	3
22	4	3	1	4	5	2
23	4	2	3	1	4	5
24	4	5	2	3	1	4
25	4	4	5	2	3	1

were prepared under a binocular magnifying glass with modified preparation needles and placed on an oblique agarized medium in such a way that the oldest anthers were situated towards the lower edge, younger ones gradually higher up and the youngest ones were placed at the upper edge. Glass tubes sized 65 x 16 x 1.2 mm with the medium were stored for 3 weeks in darkness at a temperature of about 26°C; then the tubes were transferred into light with the light regime of 16 hr day and a temperature of $28 \pm 2^\circ\text{C}$, and 8 hr night and a temperature of $24 \pm 1^\circ\text{C}$. After 76 days from inoculation, the anthers were passaged on the identical media.

Results and Discussion: No difference was observed between the cold

pre-treatment and the immediate application of anthers. More pronounced callogenesis was recorded in the following lines and types of media: L-20 on media 5, 6, 14, and 20; L-21 on media 2, 3, 4, 5, 9, 12, 18, and 20; L-23 on media 2 and 14. The most intensive growth of the callus was recorded in the first subculture at the highest KIN and IAA levels. Cytological analysis of the calli has not been carried out so far, and regeneration from covering anther layers cannot be excluded. The experiments are proceeding. For more details see Kadlec et al. (1990).

Table 2. Variable factors (mg/l).

Levels of factors:	1	2	3	4	5
1. KNO ₃	-	100	299	300	400
KCl	300	225	150	75	-
2. CaCl ₂ · 2H ₂ O	100	125	150	175	200
3. (NH ₄) ₂ SO ₄	-	67	134	201	268
4. BiG ₂ PO ₄	-	40	80	120	160
5. Kinetin	1	1.5	2	2.5	3
6. 2,4,D	0.1	0.2	0.3	0.4	0.5

Stable media components : KNO₃ 800 mg/l
 (NH₄)₂HPO₄ 270 mg/l
 KCl 75 mg/l
 MgSO₄ · 7H₂O 375 mg/l
 NaCl 57 mg/l
 Sucrose 30,000 mg/l
 Difco-agar 6,000 MG/l
 pH = 5.6

Vitamins - 1 ml. composition: B₁ 1.2 mg/l
 B₆ 5 mg/l
 PP 1 mg/l
 C 2.2 mg/l
 Glycine 3 mg/l
 Glutamine 75 mg/l
 Inositol 100 mg/l

Microelements - 1.2 ml/l

Composition: M₂BO₃ 4 mg/l
 MnSO₄ · 7H₂O 10 mg/l
 ZnSO₄ · 7H₂O 3 mg/l
 KJ 0.4 mg/l
 CuSO₄ · H₂O 0.015 mg/l
 CoCl₂ · 6H₂O 0.015 mg/l
 NaMoD₄ · 2H₂O 0.1 mg/l

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Miroslac Kadlec

Jana Suchomelova

Mendeleum, Research Centre of Genetics
Univ. Agric., Brno, 691 44 Lednice
Czechoslovakia

Vitalij Alexejevic Smirnov

Sovetova Ludmila Nikolajevna

Institut of Ecological Genetics
AS MSSR 277 000 Kishinev UdSSR

3) Response of various explants of soybean to the group of growth media with absent exogenous phytohormones.

Introduction: The objective was to reach the complete entirety of the F₀ generation plant and seed from the soybean explants without inducing it by exogenous phytohormones. The stated objective drew on a working hypothesis that had presumed that the plant cell, the tissue, is capable--by means of its own internal potential--of attaining complete regeneration of the plant integrity even without induction by the exogenous phytohormones, and of reaching the generative stage of development provided that all vital substances are available within the closest environment (Kadlec et al., 1990).

Materials and Methods: The L-23 line from the Mendeleum gene pool was used along with the following six types of media: B 5/1: Bayley et al., 1972; W: White, 1989; L: Linsmaier and Skoog, 1989; MS: Murashige and Skoog, 1962; SH: Schenk and Hildebrandt, 1972; H: Heller, 1989. Root tips, hypocotyls, epicotyls, nodes, leaf tips and apices were taken from plants cultivated in aseptic conditions. The cultivation took place in small glass tubes 65 x 16 x 1.2 mm under the light regime of 18 hr day (28 ± 1°C) and 8 hr night (24 ±

1°C). Response of the explants was evaluated after 21 days (see Table 1, Diagram 1, and Fig. 1.)

Results and Discussion: Strong elongation growth was observed in the root tips (with the exception of the W and LS media). The hypocotyl segments showed callogenesis and rhizogenesis on the B 5/2, LS, MS and SH media. The segments were largely cracked where spontaneous callogenesis occurred. The largest increase in biomass was recorded on the LS medium. In the model, both shoots and roots were initiated and seven plants were obtained in total (of which three were on the B 5/2 medium, one on the W medium, and three on the LS medium). The plants yielded altogether one plant on the LS medium and one plant on the W medium, which reached the stage of seed maturity. The leaf tips were swollen and bent in several places with no callogenesis being observed. Except for the W and H media, the apexes exhibited increased volume and formation of rosette-like leaves; no shoots were formed, however.

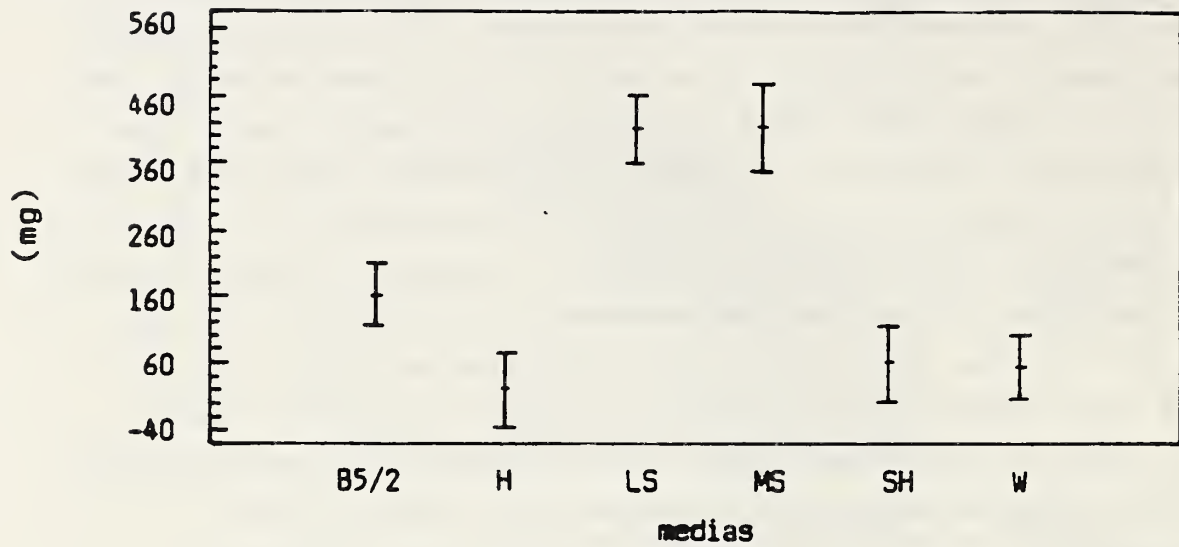
Conclusion: The hypothesis about the soybean plant reaching its complete entirety from the explants with no participation of the exogenous phytohormones was corroborated in the nodes with using the B 5/2, W and LS media. Successfully obtained were the seeds of the T₀ generation.

Table 1. Response of soybean explants on individual media.

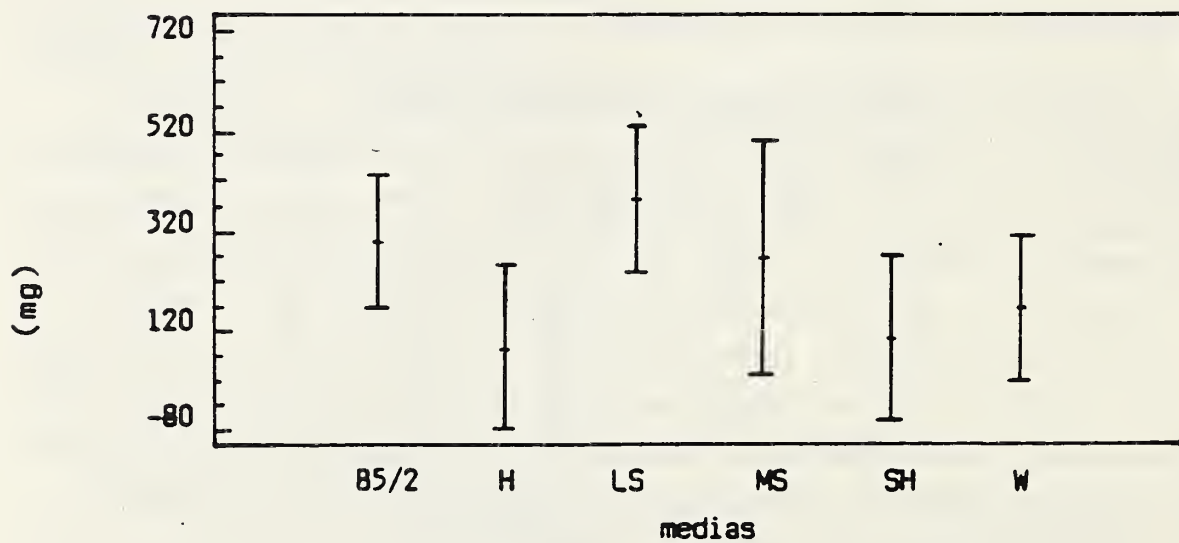
Medium/Type of explant	Root tip	Hypocotyl	Epicotyl	Node	Leaf tip	Apex
B 5/2	Pr	++R	+	P	0	P
W	0	NR	ON	PR	0	0
LS	0	++	+R	+P	0	P
MS	Pr	++	+R	+	0	P
SH	Pr	+	+N	P	0	P
H	Pr	ON	ON	0	0	0

Legend: + Callogenesis in the lower part of the explant.
 ++ Callogenesis in the cracks of the explant.
 N Browning, explant necrosis.
 0 No significant response.
 P Initiation of the first leaves or shoots.
 PR Root tip elongation.
 R Rhizogenesis.

Weight of epicotyl's explants



Weight of hypocotyl's explants

Graph 1. Weight of explants ($\bar{x} \pm 5$)

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M. Kadlec

J. Suchomelova

J. Kruzova

P. Dominik

4) Multiplication induction of shoots in *Glycine max*.

Introduction: The aim of our research was to induce immature embryos and mature seeds into producing multiple shoots for the purpose of increasing the multiplication ratio from somatic embryogenesis (Holleinova and Suchomelova, 1988). Kartha et al. (1981) mentioned regeneration from apices, Barwale et al. (1986b) from buds of cotyledons nodes, and Griga and Hejtmankova (1990) from apices and cotyledon nodes.

Materials and Methods: The trials were carried out on lines L-20, L-21, and L-23 from the gene pool of Mendeleeum. From plants cultivated in sterile conditions, explants of hypocotyl, epicotyl, buds with cotyledon and stem nodes and apices. The cultivation medium (Table 1) contained various concentrations of exogenous phytohormones (BAP 1; 5; 10 and 0 mg/l NAA: 0, 0.1; 0.5 and 1 mg/l). In this way, 12 variants were obtained. Variant No. 13 was the control (Table 2). The explants were cultivated under the following conditions: 16 hr day with $28 \pm 1^\circ\text{C}$ and 8 hr night with $24 \pm 1^\circ\text{C}$.

Evaluations were carried out after the sixth and ninth weeks. At the same time, the material was transplanted twice onto identical but fresh media. Shoots initiated by those times were separated and passed on an MSO medium.

Results and Discussion: Production of multiple shoots was observed only in explants from organized meristems. In hypocotyl and epicotyl explants there appeared only callogenesis, partly rhizogenous (Fig. 1), later developing into necrosis and subsequent death. Table 2 shows the level of initiation of

Table 1. Basic medium for multiplication

KNO ₃	2000 mg/l
MH ₄ NO ₃	320 mg/l
NaH ₂ PO ₄	270 mg/l
CaCl ₂ . 2H ₂ O	440 mg/l
MgSO ₄ . 7H ₂ O	375 mg/l
Fe-chellate	6 ml/l
Vitamins	1 ml/l
Microelements	1 ml/l
Saccharose	30 g/l
Difco-agar	6 g/l
pH = 5.6	

Table 2. Response of soybean explants (SN=stem nodes, CN= cotyledon nodes, A=apex) to multiplication induction (+ = production of a number of shoots; - = no response; 0 = necrosis and death) according to individual concentration of NAA:BAP (mg/l), lines and types of explant after the sixth and ninth weeks of cultivation.

Variety	NAA : BAP	Lines	After six weeks				After nine weeks		
			SN	CN	A	SN	CN	A	
1	0 : 1	L-20	+	+	-	+	+	0	
		L-21	+	-	-	0	0	0	
		L-23	+	-	-	+	0	0	
2	0.1 : 1	L-20	+	-	-	+	0	+	
		L-21	-	-	-	0	0	0	
		L-23	-	-	-	0	0	0	
3	0.5 : 1	L-20	+	-	+	+	+	0	
		L-21	-	-	-	0	0	0	
		L-23	-	-	-	0	0	0	
4	1 : 1	L-20	+	-	-	+	0	0	
		L-21	-	-	-	0	0	0	
		L-23	+	-	-	0	0	0	
5	0 : 5	L-20	-	+	-	+	+	0	
		L-21	+	+	-	0	0	0	
		L-23	+	-	+	0	0	+	
6	0.1 : 5	L-20	+	-	-	+	0	0	
		L-21	+	-	-	0	0	0	
		L-23	+	-	+	0	0	0	
7	0.5 : 5	L-20	-	-	+	+	0	0	
		L-21	-	-	-	0	0	0	
		L-23	+	-	-	0	0	0	
8	1 : 5	L-20	-	-	-	0	0	0	
		L-21	-	+	+	0	0	0	
		L-23	-	+	-	0	0	0	
9	0 : 10	L-20	+	-	-	+	0	0	
		L-21	-	-	-	0	0	0	
		L-23	+	-	+	+	0	0	
10	0.1 : 10	L-20	-	-	-	+	0	0	
		L-21	-	-	-	0	0	0	
		L-23	+	-	-	+	0	0	
11	0.1 : 10	L-20	-	-	+	0	0	0	
		L-21	-	-	-	0	0	0	
		L-23	-	-	-	0	0	0	
12	1 : 10	L-20	-	-	-	0	0	0	
		L-21	+	-	-	0	0	0	
		L-23	-	+	-	0	0	0	
13(K)	0 : 0	L-20	-	-	-	0	0	0	
		L-21	-	-	-	0	0	0	
		L-23	-	-	-	0	0	0	
Total		17	6	8	14	4	4		
from lines		L-20	6	2	3	9	3	1	
		L-21	4	2	2	0	0	0	
		L-23	7	2	3	5	1	3	

multiple shoots in relation to the factors that were assessed. It is obvious that the ability to form shoots was singularly dependent upon the type of cultivar or explant. The dependence upon the level of treatment (increasing or decreasing concentrations of BAP and NAA) shows no clear tendency.

Conclusion: Production of multiple shoots was obtained. Best results showed in explants of stem nodes and in line L-20. No clear statement is possible as to the impact of various concentrations of exogenous factors (BAP and NAA). The trials are continuing at various levels of pre-treatment with BAP.

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M. Kadlec
J. Suchomelova
J. Kruzova
P. Dominik

5) Hybridization in Glycine max.

Introduction: It is needless to say that crossing soybean still has a major role for creation of new varieties. This is corroborated by the fact that most new varieties that have been introduced recently in the world are of hybrid origin (Lescenko et al., 1985). Unlike some of our literary sources, which describe in detail crossing procedures, the efficiency in our obtaining hybrids is rather low under present conditions. Despite some obvious progress, our success rate is still not adequate to the efforts we make.

Materials and Methods: During the years 1979 to 1990 a series of techniques was tested in pollination with castration or without it (Fehr, 1980; Kadlec, 1988 and 1989). At the same time we analyzed the disposition of parents for crossing, for being mother or father, the influence of years, germination and viability of pollen (Kadlec et al., 1980). More than 100 parental forms were tested at one time or another. We present here a synopsis of the results in the last five years. The signs are short indications for the following expressions:

- (a) - crossing in field conditions
- (b) - crossing in greenhouse
- (1) - number of crossed flower buds
- (2) - number of pods obtained
- (3) - number of seeds
- (4) - average number of seeds in pod
- (5) - relative success rate in crossing derived from (2) and (1) in %
- (6) - number of F_1 hybrid plants (proven beyond doubt).

Results and Discussion: The total results are presented in Table 1.

From there we derive the following statements:

1. The volume of flower buds for crossing (1) according to (a) and (b) was continuously reduced down to the value that was 2.89 times lower according to (a), or 7.34 lower according to (b), respectively.
2. The number of pods obtained (2) (= the number of flower buds) is certainly a function of the skill of the hybridizor and so this value is to be taken cum grana salis. It is obvious, though, that more and more positive results were obtained in the course of time, for according to (a) a value 4.05 times higher was reached, according to (b) 1.96 higher.
3. The manual skill of the hybridizors was shown obviously in that less

damage was done to flower buds, as the average number of seeds in pod (4) increased from the original 1.74 to 2.09, according to (a), or from 1.69 to 1.96, according to (b).

4. The relative success rate in crossing, according to (b), was collectively higher (15.73%) than according to (a), which was 11.83%.

5. The number of hybrid plants that could be proved as such without any doubt (6) showed an increase, which was the most marked in 1988. In that year the real efficiency amounted to 1.15% according to (a), and 0.44% according to (b). The results of the crossing in 1989 tested in 1990 are not given. They would be too distorting and therefore not objective, because there was extensive damage to the stand, due to drought.

Conclusion: Despite some progress in success rate, which could be demonstrated, the total efficiency reached is still very low. For this reason we decided to test the crossing in vitro.

Summary: The experiments, the aim of which was to test the possibility of using explant techniques in hybridization of Prunus armeniaca L., were carried out in the laboratory of the Department of Fruticulture and Viniculture (Brno University of Agriculture) in the years 1987 to 1990.

Germinating of embryos and their subsequent growth into plants depends not only on the nutrition media but also on other influences inhibiting the germination.

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Table 1. Crossing results - synoptic evaluation according to years.

		(1)	(2)	(3)	(4)	(5)	(6)
1985	a	3353	204	354	1,74	6,08	1
	b	-	-	-	-	-	-
1986	a	1833	140	247	1,76	7,64	5
	b	961	86	145	1,69	8,95	2
1987	a	1484	170	342	2,01	11,46	6
	b	644	146	308	2,11	22,67	3
1988	a	1652	322	616	1,91	19,49	19
	b	680	125	236	1,89	18,38	3
1989	a	1161	286	595	2,08	24,63	
	b	131	23	45	1,96	17,56	

a + b		11899	1502	2888	(1,92)	12,42	39
a		9483	1122	2154	(1,92)	11,83	31
b		2416	380	734	(1,92)	15,73	8

Table 2. Response of soybean explants (SN=stem nodes, CN= cotyledon nodes, A=apex) to multiplication induction (+ = production of a number of shoots; - = no response; 0 = necrosis and death) according to individual concentration of NAA:BAP (mg/l), lines and types of explant after the sixth and ninth weeks of cultivation.

Variety	NAA : BAP	Lines	After six weeks			After nine weeks		
			SN	CN	A	SN	CN	A
1	0 : 1	L-20	+	+	-	+	+	0
		L-21	+	-	-	0	0	0
		L-23	+	-	-	+	0	0
2	0.1 : 1	L-20	+	-	-	+	0	+
		L-21	-	-	-	0	0	0
		L-23	-	-	-	0	0	0
3	0.5 : 1	L-20	+	-	+	+	+	0
		L-21	-	-	-	0	0	0
		L-23	-	-	-	0	0	0
4	1 : 1	L-20	+	-	-	+	0	0
		L-21	-	-	-	0	0	0
		L-23	+	-	-	0	0	0
5	0 : 5	L-20	-	+	-	+	+	0
		L-21	+	+	-	0	0	0
		L-23	+	-	+	0	0	+
6	0.1 : 5	L-20	+	-	-	+	0	0
		L-21	+	-	-	0	0	0
		L-23	+	-	+	0	0	0
7	0.5 : 5	L-20	-	-	+	+	0	0
		L-21	-	-	-	0	0	0
		L-23	+	-	-	0	0	0
8	1 : 5	L-20	-	-	-	0	0	0
		L-21	-	+	+	0	0	0
		L-23	-	+	-	0	0	0
9	0 : 10	L-20	+	-	-	+	0	0
		L-21	-	-	-	0	0	0
		L-23	+	-	+	+	0	0
10	0.1 : 10	L-20	-	-	-	+	0	0
		L-21	-	-	-	0	0	0
		L-23	+	-	-	+	0	0
11	0.1 : 10	L-20	-	-	+	0	0	0
		L-21	-	-	-	0	0	0
		L-23	-	-	-	0	0	0
12	1 : 10	L-20	-	-	-	0	0	0
		L-21	+	-	-	0	0	0
		L-23	-	+	-	0	0	0
13(K)	0 : 0	L-20	-	-	-	0	0	0
		L-21	-	-	-	0	0	0
		L-23	-	-	-	0	0	0
Total			17	6	8	14	4	4
from lines		L-20	6	2	3	9	3	1
		L-21	4	2	2	0	0	0
		L-23	7	2	3	5	1	3

COMENIUS UNIVERSITY

Department of Genetics and Molecular Biology

Mlynska dolina, pav. B - 1

842 15 Bratislava

Czechoslovakia

1) Correlation coefficients analysis in quantitative traits of M2 generation after laser unit and ethylenimine treatment in soybean.

Introduction: Study of correlation coefficients in quantitative traits helps the breeders determine relationships in quantitative traits and especially relationships between quantitative traits and seed yield. In our study changes of relationships in 12 quantitative traits (seed yield included) after laser unit (LU) and ethylenimine (EI) in M2 generation were observed and analyzed. Malik (1982) found out that quantitative traits such as number of pods per plant, 100-seed weight, and height of plants are significantly positively correlated with seed yield. Salehuzzaman (1979) stated positive correlation coefficients between seed yield and number and weight of pods per plant and number of seeds per plant. Path coefficient analysis showed positive influence of dry matter of plants, number of pods, and seeds per plant, and 100-seed weight on seed yield on phenotypical and genotypical level. Descescu (1982) determined the sequence of quantitative traits according to the importance of participation in seed yield as follows: the height of plants, number of nodes, and pods per plant. Accord to Ecochard (1982) the seed yield is strongly correlated with numbers of pods per plant, weight of mature plants, and the diameter of stem at the soil level. Seed weight was approximately up to 50% of the plant mature weight. Number of pods and diameter of stem at the soil level are mentioned as the most reliable indicators for high yielding genotypes. Board (1985) studied correlations of quantitative traits and seed yield and found out that the seed yield per plant was more strongly correlated with the seed yield on branches than on the seed yield on the main stem. Kokubun (1988) considered the leaf area as the most important trait in soybean in dependence upon the yield per plant. When plants are overshadowed during the period of flowering and beginning of pod

formation, it will lead to significant decrease in the number of pods and in consequence to a decrease in yield. The effect of assimilation was significantly higher in the upper leaves, in comparison with middle or lower leaves. Existence of two sources of assimilates was observed: assimilates from middle and upper leaves are streamed into pods located in the upper part of the plant; assimilates from the lower leaves and branches are streamed into pods in the lower part of the plants.

Material and Methods: Dry seeds of two varieties, 'Aida' and 'Nadneprjanskaja' were treated with helium neon laser units for 300 seconds, and EI (0.025 and 0.05%) for 24 hr at 20°C. LU were used before EI. The M1 generation was sown in the field with a row spacing of 35 cm, and in-row spacing of 5 cm. The M2 generation was sown with a row spacing of 20 cm, and in-row spacing of 3 cm. One hundred fifty plants of each treated variant, including check, were statistically analyzed for 12 quantitative traits.

Results and Discussion: Correlation coefficients in 12 traits in the M2 generation after LI and EI treatment were observed. Strongly correlated couples of quantitative traits were found, as follows: seed weight on the main stem with number of nodes; pods and seeds on the main stem and the same traits on branches; this corresponds with results of Malik (1982), Salehuzzaman (1979) and Ecochard (1982). The next pair of traits strongly correlated are: number of pods on the main stem with number of nodes, and seeds on the main stem and on branches the same couples of traits. Correlation between seed weight on the main stem and on branches is positive middle range in both varieties. The greater part of correlation coefficients of number of sterile nodes per plant with other traits are negative. The greatest positive shift of correlation coefficient values in comparison of variants treated to check was between seed weight on branches and sterility on branches in variety Nadneprjanskaja ($\Delta K < 0.35, 0.93$). Quantitative traits such as number of fertile nodes, number of pods and seed on the main stem as well as on branches are considered to be the most important quantitative traits in forming high yields in our soybean trials.

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Number of sterile nodes		
	Aida	Nadneprjanska
	ΔK	ΔK
NB	<-0.38, -0.08>	<-0.27, 0.07>
NPMS	<-0.44, -0.15>	<-0.50, -0.06>
NNMS	<-0.54, -0.25>	<-0.51, 0.01>
NPB	<-0.40, -0.15>	<-0.31, 0.18>
NNB	<-0.39, -0.14>	<-0.31, 0.09>
NSMS	<-0.40, -0.17>	<-0.50, -0.05>
SMS	<-0.29, 0.06>	<-0.32, 0.01>
NSB	<-0.38, -0.07>	<-0.30, 0.06>
SB	<-0.27, -0.09>	<-0.26, -0.06>
WSMS	<-0.39, -0.20>	<-0.49, -0.04>
WSB	<-0.35, -0.05>	<-0.28, 0.03>

Number of branches		
	Aida	Nadneprjanska
	ΔK	ΔK
NSN	<-0.38, -0.08>	<-0.27, 0.07>
NPMS	< 0.19, 0.40>	< 0.21, 0.41>
NNMS	< 0.06, 0.30>	< 0.13, 0.35>
NPB	< 0.41, 0.91>	< 0.75, 0.91>
NNB	< 0.43, 0.95>	< 0.79, 0.94>
NSMS	< 0.25, 0.45>	< 0.25, 0.44>
SMS	<-0.02, 0.08>	< 0.03, 0.18>
NSB	< 0.35, 0.84>	< 0.65, 0.85>
SB	< 0.35, 0.71>	< 0.49, 0.84>
WSMS	< 0.24, 0.42>	< 0.29, 0.52>
WSB	< 0.32, 0.83>	< 0.68, 0.90>

Number of pods on main stem		
	Aida	Nadneprejanska
	ΔK	ΔK
NSN	<-0.44, -0.15>	<-0.50, -0.06>
NB	< 0.19, 0.40>	< 0.21, 0.41>
NNMS	< 0.79, 0.98>	< 0.87, 0.97>
NPB	< 0.34, 0.53>	< 0.24, 0.39>
NNB	< 0.30, 0.54>	< 0.19, 0.39>
NSMS	< 0.83, 0.96>	< 0.88, 0.93>
SMS	< 0.41, 0.58>	< 0.45, 0.68>
NSB	< 0.35, 0.54>	< 0.12, 0.41>
SB	< 0.13, 0.47>	< 0.10, 0.34>
WSMS	< 0.82, 0.94>	< 0.83, 0.91>
WSB	< 0.34, 0.52>	< 0.12, 0.48>

Number of nodes on main stem		
	Aida	Nadneprejanska
	ΔK	ΔK
NSN	<-0.54, -0.25>	<-0.51, 0.01>
NB	< 0.06, 0.30>	< 0.13, 0.35>
NPMS	< 0.79, 0.98>	< 0.87, 0.97>
NPB	< 0.28, 0.51>	< 0.19, 0.42>
NNB	< 0.23, 0.46>	< 0.14, 0.43>
NSMS	< 0.76, 0.92>	< 0.76, 0.93>
SMS	< 0.32, 0.53>	< 0.48, 0.59>
NSB	< 0.33, 0.44>	< 0.07, 0.41>
SB	< 0.07, 0.43>	< 0.05, 0.37>
WSMS	< 0.75, 0.91>	< 0.74, 0.92>
WSB	< 0.32, 0.40>	< 0.08, 0.42>

Number of pods on branches		
	Aida	Nadneprejanska
	AK	AK
NSN	<-0.40, -0.15>	<-0.31, 0.18>
NB	< 0.41, 0.91>	< 0.75, 0.91>
NPMS	< 0.34, 0.54>	< 0.24, 0.39>
NNMS	< 0.28, 0.51>	< 0.19, 0.42>
NNB	< 0.95, 0.99>	< 0.91, 0.99>
NSMS	< 0.38, 0.84>	< 0.25, 0.43>
SMS	< 0.03, 0.24>	<-0.04, 0.19>
NSB	< 0.92, 0.98>	< 0.85, 0.97>
SB	< 0.50, 0.82>	< 0.67, 0.85>
WSMS	< 0.40, 0.65>	< 0.29, 0.45>
WSB	< 0.81, 0.95>	< 0.80, 0.93>

Number of nodes on branches		
	Aida	Nadneprejanska
	AK	AK
NSN	<-0.39, -0.14>	<-0.31, 0.09>
NB	< 0.43, 0.95>	< 0.79, 0.94>
NPMS	< 0.30, 0.54>	< 0.19, 0.39>
NNMS	< 0.23, 0.46>	< 0.14, 0.43>
NPB	< 0.95, 0.99>	< 0.91, 0.99>
NSMS	< 0.34, 0.63>	< 0.25, 0.43>
SMS	< 0.03, 0.26>	<-0.11, 0.17>
NSB	< 0.87, 0.95>	< 0.82, 0.99>
SB	< 0.48, 0.86>	< 0.61, 0.81>
WSMS	< 0.34, 0.63>	< 0.27, 0.45>
WSB	< 0.80, 0.92>	< 0.77, 0.97>

Number of seeds on main stem		
	Aida	Nadneprejanska
	AK	AK
NSN	<-0.40, -0.17>	<-0.50, -0.05>
NB	< 0.25, 0.45>	< 0.25, 0.44>
NPMS	< 0.83, 0.96>	< 0.88, 0.93>
NNMS	< 0.76, 0.92>	< 0.76, 0.93>
NPB	< 0.38, 0.64>	< 0.25, 0.43>
NNB	< 0.34, 0.63>	< 0.25, 0.43>
SMS	< 0.15, 0.39>	< 0.20, 0.48>
NSB	< 0.39, 0.64>	< 0.14, 0.48>
SB	< 0.16, 0.42>	< 0.14, 0.37>
WSMS	< 0.94, 0.97>	< 0.95, 0.97>
WSB	< 0.38, 0.61>	< 0.15, 0.54>

Sterility on main stem		
	Aida	Nadneprejanska
	AK	AK
NSN	<-0.29, 0.06>	<-0.32, 0.01>
NB	<-0.02, 0.08>	< 0.03, 0.18>
NPMS	< 0.41, 0.58>	< 0.45, 0.68>
NNMS	< 0.32, 0.53>	< 0.48, 0.59>
NPB	< 0.03, 0.24>	< 0.04, 0.19>
NNB	< 0.03, 0.26>	<-0.11, 0.17>
NSMS	< 0.15, 0.39>	< 0.20, 0.48>
NSB	< 0.02, 0.24>	<-0.05, 0.15>
SB	<-0.02, 0.30>	< 0.00, 0.22>
WSMS	< 0.17, 0.37>	< 0.19, 0.50>
WSB	< 0.01, 0.29>	<-0.07, 0.16>

Number of seeds on branches		
	Aida	Nadneprejanska
	AK	AK
NSN	<-0.38, -0.07>	<-0.30, -0.06>
NB	< 0.35, 0.84>	< 0.65, 0.85>
NPMS	< 0.35, 0.54>	< 0.12, 0.42>
NNMS	< 0.33, 0.44>	< 0.07, 0.41>
NPB	< 0.92, 0.98>	< 0.85, 0.97>
NNB	< 0.87, 0.95>	< 0.82, 0.99>
NSMS	< 0.39, 0.64>	< 0.14, 0.48>
SMS	< 0.02, 0.24>	<-0.05, 0.15>
SB	< 0.37, 0.72>	< 0.33, 0.94>
WSMS	< 0.42, 0.67>	< 0.19, 0.48>
WSB	< 0.93, 0.99>	< 0.95, 0.99>

Sterility on branches		
	Aida	Nadneprejanska
	AK	AK
NSN	<-0.27, -0.09>	<-0.25, -0.06>
NB	< 0.35, 0.71>	< 0.49, 0.84>
NPMS	< 0.13, 0.47>	< 0.10, 0.34>
NNMS	< 0.07, 0.43>	< 0.05, 0.37>
NPB	< 0.50, 0.82>	< 0.67, 0.85>
NNB	< 0.48, 0.86>	< 0.61, 0.81>
NSMS	< 0.16, 0.42>	< 0.14, 0.37>
SMS	<-0.02, 0.30>	< 0.00, 0.22>
NSB	< 0.37, 0.72>	< 0.33, 0.94>
WSMS	< 0.14, 0.56>	< 0.13, 0.35>
WSB	< 0.33, 0.70>	< 0.35, 0.93>

Seed weight on main stem			Seed weight on branches		
	Aida	Nadneprejanska		Aida	Nadneprejanska
	AK	AK		AK	AK
NSN	<-0.39, -0.20>	<-0.49, -0.04>	NSN	<-0.35, -0.05>	<-0.28, 0.03>
NB	< 0.24, 0.42>	< 0.29, 0.52>	NB	< 0.32, 0.83>	< 0.68, 0.90>
NPMS	< 0.82, 0.94>	< 0.83, 0.91>	NPMS	< 0.34, 0.52>	< 0.12, 0.48>
NNMS	< 0.75, 0.91>	< 0.74, 0.92>	NNMS	< 0.32, 0.40>	< 0.08, 0.42>
NPB	< 0.40, 0.62>	< 0.29, 0.45>	NPB	< 0.81, 0.95>	< 0.80, 0.93>
NNB	< 0.34, 0.63>	< 0.27, 0.45>	NNB	< 0.80, 0.92>	< 0.77, 0.97>
NSMS	< 0.94, 0.97>	< 0.95, 0.97>	NSMS	< 0.38, 0.61>	< 0.15, 0.54>
SMS	< 0.17, 0.37>	< 0.19, 0.50>	SMS	< 0.01, 0.29>	<-0.07, 0.16>
NSB	< 0.42, 0.67>	< 0.19, 0.48>	NSB	< 0.93, 0.99>	< 0.95, 0.99>
SB	< 0.14, 0.56>	< 0.13, 0.35>	SB	< 0.33, 0.70>	< 0.35, 0.93>
WSB	< 0.42, 0.66>	< 0.20, 0.54>	WSMS	< 0.42, 0.66>	< 0.20, 0.54>

AK <-0.38, -0.08> - interval of 6 correlation coefficients values - treated variants [possible combinations in LU and 2 concentrations of EI] and check.

NSN = number of sterile nodes

NB = number of branches

NPMS = number of pods on main stem

NNMS = number of fertile nodes on main stem

NPB = number of pods on branches

NNB = number of nodes on branches

NSMS = number of seeds on main stem

SMS = sterility on main stem

NSB = number of seeds on branches

SB = sterility on branches

WSMS = seed weight on main stem

WSB = seed weight on branches

L = LU not used

L* = LU was used according to Materials and Methods mentioned above

EI¹ = EI was used in concentration 0.025%

EI² = EI was used in concentration 0.05%

0 = no significant differences in correlation coefficients were observed between variants treated and check

+ = positive significant difference in correlation coefficients between variants treated and check

- = negative significant difference in correlation coefficients between variants treated and check.

2) Changes of correlation coefficients in quantitative traits in the M_2 generation after laser unit and ethylenimine treatment in soybean.

Introduction: Results in studies about influence of laser unit (LU) on plants are various. Volodin et al. (1984), Kornienko (1981) and Chochlov (1987) claim that mutants were gained after laser unit treatment in barley, wheat, sugar beet, tomatoes, etc. (however, not in soybean). Mutation effectivity after use of various types of lasers was described. But, in contrast, in soybean, no influence was observed after LU treatment (Plesnik, 1991). No significant differences in germination, survival in the M_1 and M_2 generations and chlorophyll mutants in the M_2 generation were observed after He - Ne laser units treatment in soybean. Chlorophyll mutants, morphological mutants, and mutants in quantitative traits were observed after ethylenimine (EI) treatment. Mutants mentioned above were caused by chromosomal aberrations which were cytologically investigated (Maschkin et al., 1979, 1980; Prokudina, 1979).

Material and Methods: Dry seeds of two varieties, 'Aida' and 'Nadneprijanskaja', were treated with helium neon laser units for 300 seconds, and with EI (0.025 and 0.05%) for 24 hr at 20°C. LU were used before EI. the M_2 generation was sown in the field, with 35 cm between rows and within-row spacing of 5 cm. The M_1 generation was sown with 20 cm between rows, and within-row spacing of 3 cm. One hundred fifty plants of each variant treated, including check, were statistically analyzed for 12 quantitative traits. Changes of correlation coefficients values in the M_2 generation were investigated.

Results and Discussion: Changes of correlation coefficients values in the M_2 generation will be presented. The greatest number of significant changes of correlation coefficient values in variants treated to the check was found in correlation in traits as number of sterile nodes per plant, with the other 11 traits and number of branches per plant with the other 11 traits in both varieties, as well as in seed weight on the main stem in variety Nadneprijanskaja. In contrast, the least number of changes were observed in traits such as sterility on the main stem with the other 11 traits, number of

of seeds on branches with the other 11 traits and seed weight on branches with the other 11 traits, in both varieties. No significant positive changes in correlation coefficients values were found in variety Nadneprjanskaja, in variants treated with LU and EI 0.025%. The greatest number of significant negative correlation coefficients changes was in variant treated by EI 0.025% in variety Nadneprjanskaja. In comparing the two varieties, Nadneprjanskaja was more sensitive to changes of correlation coefficients than variety Aida. Strong influence of EI and weak influence of LU on correlation coefficient changes in both soybean varieties were observed.

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Changes in correlation coefficients from the check in M2 generation

[illegible]

Changes of correlation coefficients from the check in M2 generation

[illegible]

Changes of correlation coefficients from the check in M2 generation

[illegible]

Changes of correlation coefficients from the check in M2 generation
Sterility on branches

	Aida					Nadneprijanskaja				
	L-EI ¹	L-EI ²	L ⁺ EI ⁻	L ⁺ EI ¹	L ⁺ EI ²	L-EI ¹	L-EI ²	L ⁺ EI ⁻	L ⁺ EI ¹	L ⁺ EI ²
NSN	0	0	0	0	0	0	0	0	0	0
NB	0	0	0	0	0	0	0	0	0	0
NPMS	0	+	+	0	+	0	0	0	+	+
NNMS	0	+	+	0	0	0	+	0	0	+
NPB	0	0	0	0	0	0	0	0	0	0
NNB	0	0	0	0	0	0	0	0	0	0
NSMS	0	+	+	0	+	0	+	0	0	+
SMS	0	0	0	0	0	0	0	0	0	0
NSB	0	0	0	0	0	0	0	0	0	0
WSMS	0	+	+	0	+	0	+	0	+	+
WSB	0	0	0	0	0	0	0	0	0	0

Changes of correlation coefficients from the check in M2 generation
Number of pods on the main stem

	Aida					Nadneprijanskaja				
	L-EI ¹	L-EI ²	L ⁺ EI ⁻	L ⁺ EI ¹	L ⁺ EI ²	L-EI ¹	L-EI ²	L ⁺ EI ⁻	L ⁺ EI ¹	L ⁺ EI ²
NSN	-	-	0	-	0	0	+	0	0	+
NB	0	0	+	0	+	0	0	0	+	0
NNMS	0	0	0	0	0	0	0	0	0	0
NPB	0	0	0	0	0	-	-	-	0	0
NNB	0	0	0	-	0	-	0	-	0	0
NSMS	0	0	0	0	0	0	0	0	0	0
SMS	0	0	0	0	0	0	0	0	0	0
NSB	0	0	0	0	0	-	0	-	0	0
SB	-	0	0	-	0	0	0	0	+	+
WSMS	0	0	0	0	0	0	0	0	0	0
WSB	0	0	0	0	0	-	0	-	0	0

Changes of correlation coefficients from the check on M2 generation
Number of fertile nodes on the main stem

	Aida					Nadneprijanskaja				
	L-EI ¹	L-EI ²	L ⁺ EI ⁻	L ⁺ EI ¹	L ⁺ EI ²	L-EI ¹	L-EI ²	L ⁺ EI ⁻	L ⁺ EI ¹	L ⁺ EI ²
NSN	-	0	0	-	0	0	+	0	0	+
NB	0	0	0	0	0	0	+	0	+	0
NPMS	0	0	0	0	0	0	0	0	0	0
NPB	0	0	0	-	0	0	+	0	0	+
NNB	-	0	0	-	0	0	+	0	0	+
NSMS	0	0	0	0	0	0	0	0	0	0
SMS	0	0	0	0	0	0	0	0	0	0
NSB	0	0	0	0	0	0	+	0	0	+
SB	0	+	+	0	0	0	+	0	0	+
WSMS	0	0	0	0	0	0	0	0	0	0
WSB	0	0	0	0	-	-	0	-	-	0

Changes of correlation coefficients from the check in M2 generation

	Number of seeds on the main stem									
	Aida					Nadneprijanskaja				
	$L^{-}EI^{-1}$	$L^{-}EI^{-2}$	$L^{+}EI$	$L^{+}EI^{-1}$	$L^{+}EI^{-2}$	$L^{-}EI^{-1}$	$L^{-}EI^{-2}$	$L^{+}EI^{-}$	$L^{+}EI^{-1}$	$L^{+}EI^{-2}$
NSN	0	0	+	0	0	0	+	0	0	+
NB	-	-	0	-	0	-	-	0	0	-
NPMS	0	0	0	0	0	0	0	0	0	0
NNMS	0	0	0	0	0	0	0	0	0	0
NPB	0	0	0	0	0	-	-	-	0	0
NNB	0	0	0	0	0	-	0	-	0	0
SMS	+	0	+	0	0	0	0	-	0	0
NSB	0	0	0	0	0	-	0	0	0	0
SB	0	+	+	0	+	0	+	0	0	+
WSMS	0	0	0	0	0	0	0	0	0	0
WSB	0	0	0	0	0	-	0	0	0	0

Changes of correlation coefficients from the check in M2 generation

[illegible]

Changes of correlation coefficients from the check in M2 generation

	Number of sterile nodes per plant									
	Aida					Nadneprijanskaja				
	L^-E1^1	L^-E1^2	L^+E1	L^+E1^1	L^+E1^2	L^-E1^1	L^-E1^2	L^+E1	L^+E1^1	L^+E1^2
NB	-	-	-	0	-	0	0	0	0	0
NPMS	-	-	0	-	0	0	+	0	0	+
NNMS	-	0	0	-	0	0	+	0	0	+
NPB	0	-	-	0	-	0	0	0	0	0
NNB	0	-	-	0	-	0	0	0	0	0
NSMS	0	0	+	-	0	0	+	0	0	+
SMS	0	0	0	0	0	0	0	0	0	0
NSB	+	0	0	+	0	0	0	0	0	0
SB	0	0	0	0	0	0	0	0	0	0
WSMS	-	-	0	-	0	0	+	0	0	+
WSB	0	0	0	+	0	0	0	0	0	0

Changes of correlation coefficients from the check in M2 generation

[illegible]

$\Delta K < -0.38, -0.08 >$ - interval of 6 correlation coefficients values - treated variants [possible combinations in LU and 2 concentrations of EI] and check.

NSN - number of sterile nodes

NB - number of branches

NPMS - number of pods on main stem

NNMS - number of fertile nodes on main stem

NPB - number of pods on branches

NNB - number of nodes on branches

NSMS - number of seeds on main stem

SMS - sterility on main stem

NSB - number of seeds on branches

SB - sterility on branches

WSMS - seed weight on main stem

WSB - seed weight on branches

L⁻ - LU not used

L⁺ - LU was used according to Materials and Methods mentioned above

EI¹ - EI was used in concentration 0.025%

EI² - EI was used in concentration 0.05%

0 - no significant differences in correlation coefficients were observed between variants treated and check

+

 - positive significant difference in correlation coefficients between variants treated and check

-

 - negative significant difference in correlation coefficients between variants treated and check.

Changes of correlation coefficients from the check in M2 generation

	Seed weight on the main stem									
	Aida					Nadneprijanskaja				
	$L^{-}EI^1$	$L^{-}EI^2$	$L^{+}EI^{-}$	$L^{+}EI^1$	$L^{+}EI^2$	$L^{-}EI^1$	$L^{-}EI^2$	$L^{+}EI^{-}$	$L^{+}EI^1$	$L^{+}EI^2$
NSN	-	-	0	-	0	0	+	0	0	+
NB	-	0	0	-	0	-	-	-	0	-
NPMS	0	0	0	0	0	0	0	0	0	0
NNMS	0	0	0	0	0	0	0	0	0	0
NPB	0	0	0	0	0	-	-	-	0	0
NNB	0	0	0	0	0	-	0	-	0	0
NSMS	0	0	0	0	0	0	0	0	0	0
SMS	+	0	+	0	0	0	0	-	0	0
NSB	0	0	0	0	0	-	0	0	0	0
SB	0	+	+	0	+	0	+	0	+	+
WSB	0	0	0	0	0	-	0	0	0	0

Changes of correlation coefficients from the check in M2 generation

	Seed weight on branches									
	Aida					Nadneprijanskaja				
	$L^{-}EI^1$	$L^{-}EI^2$	$L^{+}EI^{-}$	$L^{+}EI^1$	$L^{+}EI^2$	$L^{-}EI^1$	$L^{-}EI^2$	$L^{+}EI^{-}$	$L^{+}EI^1$	$L^{+}EI^2$
NSN	0	0	0	+	0	0	0	0	0	0
NB	0	0	0	0	0	0	0	0	0	0
NPMS	0	0	0	0	0	-	0	-	0	0
NNMS	0	0	0	-	0	-	0	-	-	0
NPB	0	0	0	0	0	0	0	0	0	0
NNB	0	0	0	0	0	0	0	0	0	0
NSMS	0	0	0	0	0	-	0	0	0	0
SMS	0	0	0	0	0	0	0	0	0	0
NSB	0	0	0	0	0	0	0	0	0	0
SB	0	0	0	0	0	0	0	0	0	0
WSMS	0	0	0	0	0	-	0	0	0	0

Svetozar Plesnik

UNIVERSITY OF RAJASTHAN

Experimental Morphogenesis Laboratory

Department of Botany

Jaipur 302 004 INDIA

1) Multiple shoot formation from cotyledonary node and shoot apex cultures of Glycine max.

We have screened 20 genotypes of soybean, viz. AKSS-60, AKSS-61, AKSS-65, AKSS-66, AMSS-23, AMSS-34, AMSS-40, AMSS-54, 'Bragg', EC-93602, EC-95268, EC-95269, EC-95272, EC-95275, Jawa-16, JS-72-44(Gaurav), MACS-13, MACS-58, PK-472, and Pusa-16, grown in India for their morphogenetic competence to produce multiple shoots from cotyledonary node and shoot apex. The result indicated that the morphogenetic competence was influenced by (i) type of genotype, (ii) hormonal regime in the culture medium, (iii) explant type and (iv) passages of subculture. Multiple shoot bud formation from the meristematic explants is particularly significant in the genetic transformation experiments utilizing the particle gun method and subsequent regeneration of transgenic plants (Christou et al., 1990).

Materials and Methods: Seeds were obtained from B.P. Singh, NBPGR, New Delhi 12 (India). They were surface-sterilized (5-10 min) in a 0.1% (w/v) HgCl_2 solution, rinsed (3-4 times) in sterile distilled water. Seeds were sown on 1/2-strength, hormone free, agar-solidified (0.6-0.8% w/v) Murashige and Skoog (1962) medium, and germinated in a culture room (continuous fluorescent illumination; 1200 lux; $26 \pm 2^\circ\text{C}$). Seven- to 15-day-old seedlings were dissected, and the cotyledonary node and shoot apex explants placed on three induction media (IM) containing B5 minerals and vitamins (Gamborg et al., 1968), 3% sucrose, 0.6% agar, and supplemented with 0.005 mg/l IBA + 1.0 mg/l BAP (IM-1), 0.005 mg/l IBA + 5.0 mg/l BAP (IM-2) and 0.005 mg/l IBA + 10.0 mg/l BAP (IM-3), respectively (Rech et al., 1988).

Results and Discussion: Multiple shoot bud induction was observed from cotyledonary node and shoot apex of all the 20 tested genotypes on all the three induction media used. However, the intensity of response varied among the genotypes. The highest number of multiple bud (>30) production was observed from the genotypes AMSS-40, EC-95272, JS-72-44, and MACS-13, on IM-1, with 40-100% cultures showing a response. Shoot bud elongation was obtained on B5 medium supplemented with IVA (0.005 mg/l + BAP (0.2 mg/l). On this medium new shoot buds also developed while older ones elongated. The shoot buds were

subcultured after every four weeks. The number of buds increased up to 100 in second and third subcultures on IM-1. Long shoots (3.5 cm) were rooted on 1/2 strength, hormone-free B5 medium containing 6% sucrose.

In our experiments, out of 20 tested genotypes, AMS-40, EC-95272, and MACS-13 were the most responsive for multiple bud formation from the cotyledonary node, while shoot apex of AMSS-40 gave best response (Sharma and Kothari, 1991). IM-1 was the most effective hormonal condition that evoked the highest number of shoot buds with little callusing. However, IM-2 and IM-3 evoked a number of buds in initial cultures but in the successive passages only callusing was favored. In the second passage most of the genotypes induced more than 15 buds on IM-1. Genotype AMSS-40 produced up to 100 shoot buds in the second passage from both the explants (Sharma et al., 1991). Best shoot bud elongation and rooting was obtained on half-strength, hormone-free B5 medium containing 6% sucrose. It is hoped that formation of multiple shoots from cotyledonary nodes and shoot apices of G. max will prove valuable in future transformation studies using particle gun method and subsequent regeneration of transgenic plants.

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S. L. Kothari

N. Chandra

V. K. Sharma

NATIONAL RESEARCH CENTRE FOR SOYBEAN (ICAR)

Indore 452 001 M.P.

INDIA

1) Genetics of pod shattering in soybean.

Pod shattering in soybean, prior to, as well as during, harvest, causes yield losses. In tropical and subtropical countries, the losses are severe. Soybean cultivars vary in their inherent capacity to manifest pod shattering (Tiwari and Bhatnagar, 1988, 1991). Information available on genetics of shattering in soybean is scanty, probably owing to the absence of discrete classes in filial generations and difficulty in estimation of the shattering behavior of genotypes. This report contains information on gene action for pod shattering in soybean.

Three soybean lines, namely 'Bragg' (a shattering-resistant variety), 'PK-472' (moderately resistant/intermediate reaction), and 'NRC-1' (highly vulnerable to shattering), were crossed in all possible combinations, excluding reciprocals, with three testers, namely 'JS-71-05', 'Guarav' and 'Punjab-1', representing the commercially grown varieties of the predominant soybean belt of central India, where pod shattering is a serious problem. The F₁'s were grown in replicated rows. Ten representative pods from three to five hybrid plants from each of the three replications were carefully detached at harvest maturity (13-14% seed moisture). The detached pods were kept in brown papers and allowed to equilibrate for a week at room temperature. Thereafter, the detached pods were subjected to oven-drying at 40°C for 24 hours. Percentage of pod shattering thus induced was recorded. This method was earlier ascertained to reliably predict the shattering behavior of soybean genotypes and give high correlation with field shattering. The shattering data were analyzed for combining ability and gene action as per Kempthorne (1957). The parents were included in the analysis for comparison. Average degree of dominance was estimated according to Comstock and Robinson (1952).

The mean values obtained for pod-shattering percentage in case of parents and hybrids are given in Table 1. All the hybrids exhibited a significant degree of shattering, even in cases where one of the parents was resistant and did not show any pod shattering. This indicated that susceptibility to pod shattering could be dominant or partially dominant to shattering resistance.

The analysis of variance showed highly significant differences for treatments, parents, crosses, and parents vs. crosses interaction (Table 2). Analysis of variance for combining ability showed highly significant differences for line vs. tester interaction (Table 2). Variance for general combining ability (σ gca) was found to be more than the variance for specific combining ability (σ sca). It showed the preponderance of additive gene action for pod shattering. It was, however, noteworthy that the magnitude of difference between σ gca (64.81) and σ sca (47.22) was not high and, although additive gene action was predominant in determining the character, the dominant gene action also played its role. This fact is further borne out by the average degree of dominance (\bar{a}) which was estimated to be 1.7 for the character.

The combining ability effects obtained in the present study are given in Table 3. Since we measured shattering intensity, and not the resistance to shattering, the combining ability effects with negative values, meaning less shattering, were considered desirable. Variety Bragg, a resistant type, was found to be the best general combiner (-9.6) among lines. Among testers, variety JS-71-05 was found to be the best general combiner (-7.41), with its combining ability effects next only to Bragg. Varieties Punjab-1 and NRC-1 were found to be the most undesirable general combiners.

The present study reveals the following:

- (i) Susceptibility to pod shattering in soybean is dominant/partially dominant to resistance. This confirms the earlier report of Tsuchiya and Sunada (1979). Further, crosses between the wild Glycine soja Sieb and Zucc., highly vulnerable to pod shattering, and the cultigen are known to give hybrids that show pod shattering and it necessitates three to four backcrosses before the pod shattering could be got rid of (Carpenter and Fehr, 1986).
- (ii) σ gca (64.81) for pod shattering was found to be more than σ sca (47.32) indicating a preponderance of additive gene action although dominant gene action also played an important role in expression of the character. The average degree of dominance was 1.7
- (iii) Varieties Bragg and Js-71-05 were identified to be good general combiners. These could be used as donors in breeding programs. Varieties Punjab-1 and NRC-1 were identified to be the most undesirable general combiners among the material studied.

Further studies, especially of the F₂ generations obtained from different combinations, are warranted.

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Table 1. Mean performance of parents and F₁'s for pod shattering (%) in soybean.

Lines	Testers		
	JS 71-05 (33.33)	Gaurav (46.67)	Punjab-1 (66.67)
Bragg (0.00)	46.67	43.33	56.67
PK-472 (36.67)	50.00	63.33	60.00
NRC-1 (86.67)	56.67	63.33	86.67

Values in parentheses give mean performance of parents (lines/testers).

Table 2. Analysis of variance for combining ability for pod shattering in soybean.

Source	df	MSS
Replications	2	62.22
Treatments	14	1368.89**
Parents	5	2663.33**
Crosses	8	484.26**
Parents vs. crosses	1	1973.70**
Lines	2	903.70
Testers	2	648.15
Lines vs. testers	4	192.59**
Error	28	45.56
σ gca	64.81	
σ sca	47.22	
σ i.e.	1.71	
$\sigma^2 = \sigma^2 A + \sigma^2 D + \sigma^2 A \times D$		

*** Significant at 0.01 P.

Table 3. Estimates of general and specific combining ability effects for pod shattering in soybean.

General combining ability effects		Specific combining ability effects	
Parents	Estimates	Crosses	Estimates
Lines			
Bragg	-9.60	Bragg X JS 71-05	5.18
PK-472	-0.70	Bragg X Gaurav	-3.70
NRC-1	10.40	Bragg X Punjab-1	-1.48
Testers			
JS 71-05	-7.41	PK-472 X JS 71-05	-0.37
Gaurav	-1.85	PK-472 X Gaurav	7.41
Punjab-1	9.26	PK-472 X Punjab-1	-7.04
		NRC-1 X JS 71-05	-4.81
		NRC-1 X Gaurav	-3.70
		NRC-1 X Punjab-1	8.52

S.P Tiwari

P.S. Bhatnagar

JAPAN

KYUSHU UNIVERSITY

Faculty of Agriculture

6-10-1, Hakozaki, Higashi-ku

Fukuoka 812 Japan

1) Inheritance and growth habits of two soybean dwarf lines.

Dwarf or semi-dwarfness in rice and wheat is one of the most important breeding objectives because these characters often accompany high harvest index, lodging resistance and thereby good adaptability to heavy fertilizer application. But, up to now, there are no useful cultivars with dwarf character in soybean. This report describes the inheritance and growth habits of two dwarf lines, Hyuga-dwarf line and PI 227,224, comparing with other Japanese late maturing cultivars.

1. Hyuga-dwarf line

(a) Origin. Hyuga-dwarf line is a dwarf mutant induced from Hyuga, late cultivar in Kyushu area, by the application of ethyleneimine in 1975 by Dr. Shojiro Shida, ex-prof of Miyazaki University.

(b) Growth habits. Hyuga-dwarf line showed slower vegetative growth than Hyuga, and had short internodes, vigorous branches from lower nodes and small and deep green lamina. Hyuga-dwarf line had advantageous distribution of dry matter as a high seed-stem weight ratio. Under common cultural conditions in the Kyushu area, Hyuga-dwarf line reached flowering and maturity about seven days later and ten days later than Hyuga, respectively. Some plant characters of Hyuga-dwarf line are shown in Table 1.

(c) Inheritance. In order to clarify inheritance of the dwarf character in Hyuga-dwarf line, reciprocal crosses were made between Hyuga-dwarf line and its original cultivar Hyuga in 1984. In 1986, F_2 plants were grown. For reference, a cross was made between Hyuga-dwarf line and Fukuyutaka, leading cultivar in the Kyushu area.

Segregation of F_2 plants is shown in Table 2.

From these results, it was clear that the dwarf character of Hyuga-dwarf

line was expressed by one recessive major gene.

2. PI 227,224

(a) Origin. PI 227,224 used in this experiment was supplied from the laboratory of breeding of Kyushu University. The origin of PI 227,224 is a Japanese cultivar 'Yahagi', which was introduced from Kyoto, Japan, by the USDA in 1956 (Hartwig and Edwards, 1975).

Eight entries with the name Yahagi in the National Institute of Agrobiological Resource, Tsukuba, Japan, were grown and observed. Judging from growth habits and plant characteristics, it was found there were five cultivars which might be identical to PI 227,224. Now, identifying the dwarf gene is under way. A normal type of Yahagi and PI 227,224, which might be the original cultivar of Yahagi and PI 227,224, was also identified.

(b) Growth habits. PI 227,224 showed brachytic internodes and zigzag character in stem arrangement. The main stem length was much shorter than the sum of total internode length. Zigzag ratio (the sum of total internode length/main stem length) of Yahagi (normal type) or Fukuyutaka was usually 1.01 to 1.02, whereas that of PI 227,224 was 1.04 to 1.05. Some plant characters of PI 227,224 are shown in Table 3.

(c) Inheritance. Reciprocal crosses were made between PI 227,224 and Yahagi (normal type) in 1988. In 1990, F_2 plants were grown.

Segregation of F_2 plants is shown in Table 4.

On the inheritance of dwarf character in PI 227,224, there are two opinions: one explained it with a pair of major genes (Kilen, 1977) and the other with two pair of major genes (Boerma and Jones, 1978). From these results, it was clear that the dwarf character of PI 227,224 was expressed by one recessive major gene.

3. Double dwarf line

(a) Crossing between Hyuga-dwarf line and PI 227,224. Reciprocal crosses were made between Hyuga-dwarf line and PI 227,224 in 1986 and 1987.

All F_1 plants expressed normal character in plant type, showing that the dwarf gene of Hyuga-dwarf line was different from that of PI 227,224.

(b) Segregation of F_2 plants. In 1988 and 1989, F_2 plants were grown. Segregation of F_2 plants is shown in Table 5. Dwarf plants could be divided into PI 227,224 type and Hyuga-dwarf type by observing the zigzag character

and leaf color.

From these results, it was clear that two dwarf genes worked independently. One sixteenth of the population had double dwarf recessive genes. The main stem length of these plants was about 20 cm, without decrease in number of nodes on the main stem.

A few lines of F_3 (1989) and F_4 (1990) generations with the double dwarf genes were planted and the plant type was observed. The dwarf character fixed in F_3 generation, whereas other characters showed variability. Double dwarf plants were too short to achieve high yield. Plant type PI 227,224 showed that the dwarf gene of PI 227,224 would be more useful than that of Hyuga-dwarf line.

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Table 1. Plant characters in Hyuga-dwarf line.

Line	Main stem length (cm)	No. of nodes on main stem	Seed weight per plant (g)	Stem weight per plant (g)	Ratio of seed/stem weight
Hyuga-dwarf	55.3	18.7	38.7	17.0	2.30
Hyuga	84.1	19.5	56.2	36.4	1.56
Fukuyutaka (check cv.)	83.9	18.8	64.8	33.2	1.95

Each value indicates the mean of two replications with 10 plants.
 All cultivars were sown 2 July, 1988.

Table 2. F₂ segregation of crosses between Hyuga-dwarf line and Hyuga.

Cross combination	<u>Phenotype frequency</u>		Ratio tested	X ² value	P
	Normal	Dwarf			
Hyuga x Hyuga-dwarf					
Observed	363	120	3:1	0.00069	0.950-0.990
Expected	362.25	120.75			
Hyuga-dwarf x Hyuga					
Observed	286	82	3:1	1.30796	0.250-0.500
Expected	276	92			
Fukuyutaka x Hyuga dwarf					
Observed	217	78	3:1	0.32655	0.500-0.750
Expected	221.25	73.75			

Table 3. Plant characters of PI 227,224.

Line	Main stem length (cm)	No. of nodes of main stem	Seed weight per plant (g)	Stem weight per plant (g)	Ratio of seed/stem weight
PI 227,224	28.1	15.4	37.0	9.6	3.85
Yahagi(N)	48.1	15.0	41.5	14.7	2.82
Fukuyutaka (check cv.)	45.2	15.4	40.7	11.3	3.60

Each value indicates the mean of two replications with 10 plants.
All cultivars were sown 6 July 1989.

Table 4. F₂ segregation of crosses between PI 227,224 and Yahagi(normal) type.

Cross combination	<u>Phenotype frequency</u>		Ratio tested	X ² value	P
	Normal	Dwarf			
Yahagi(N) x PI 227,224					
Observed	279	82	3:1	0.88734	0.750-0.900
Expected	270.75	90.25			
PI 227,224 X Yahagi(N)					
Observed	224	75	3:1	0.00115	0.950-0.990
Expected	224.25	74.75			
Fukuyutaka X PI 227,224					
Observed	133	42	3:1	0.04762	0.750-0.900
Expected	131.25	43.75			

Table 5. F_2 segregation of crosses between Hyuga-dwarf line and PI 227,224.

Cross combination	Phenotype frequency				Ratio tested
	Normal	Dwarf	Dwarf	Double	
	(Hyuga)	(PI 227,224)	(PI 227,224)	dwarf	
<hr/>					
Hyuga-dwarf x PI 227,224					
Observed	496	167	153	59	9:3:3:1
Expected	492.19	164.06	164.06	54.69	
PI 227,224 X Hyuga-dwarf					
Observed	548	185	203	66	9:3:3:1
Expected	563.63	187.88	187.88	62.63	
Hyuga-dwarf X PI 227,224	X^2 value	1.16744		P 0.750-0.990	
PI 227,224 X Hyuga-dwarf	X^2 value	1.87582		P 0.500-0.750	

Hyuga-dwarf and PI 227,224 types were classified by observing the zigzag character and the leaf color.

T. Umezaki

POLAND

AGRICULTURE UNIVERSITY

Faculty of Agriculture

Department of Genetics and Plant Breeding

60-625 Poznan ul. Wojska Polskiego 71C

POLAND

1) Heterosis effect in the cool climate of Wielkopolska region of Poland.

Some heterosis effects were reported by Brim and Cockerham (1961), Chaudhary and Singh (1974), Paschal and Wilcox (1975) and Kunta et al., (1985). Konieczny (1986) showed that, in the Wielkopolska region, conditions (cool summer) the heterosis effects were distinctively great. Parental genotypes in Polish crosses were selected directly from the USDA collection. Heterosis effect was calculated as a comparison of the characteristics of examined F1 plants to parental forms which, because of distant origin, were not adapted to Polish conditions. This could explain the range of heterosis effects observed in the first F1 plants examined in the Wielkopolska region.

In this work, characteristics of F1 plants and their parental forms were examined. The purpose of this report was to examine F1 plants obtained by crossing advanced breeding lines selected out from previous crossing cycles (Konieczny, 1986). Such lines were better adapted to Polish conditions than PI's directly taken from the USDA collection and, therefore, might show heterosis effects different from those observed in previous crossings.

Materials and Methods: Experiment was carried out in the field at Solacz, Poznan. Materials were sown on 5 May, 1988. Distances between rows were 0.5 m and interplant spaces in the row were 0.2 m. Parental forms were sown in the same pattern in adjacent rows on both sides of F1 plants and used for calculating heterosis effects. Heterosis effects were calculated in two ways: first, by comparison of F1 plants to better (more efficient) parents, and second, comparison to the mean value of both parents. The following characteristics were recorded: vegetation period as the number of days from planting to harvest maturity, plant height, branch number, pod and seed number per plant, seed yield per plant, and 100-seed weight. Parental genotypes and their characteristics are presented in Table 1.

Results: Vegetative period. The F1 plants tended to mature later than parental forms in all of the examined cross combinations. In the '104 x 16'

cross combination, the F1 plants were shorter in the vegetative period than the mean of both parents (Table 2).

Plant height. The F1 plants in the following crosses--'2349 x Fiskeby V', '1017 x Fiskeby V', and '118 x 2336'--were shorter than parental genotypes (Table 2). The greatest heterosis effect (over 200%) was found in the cross 'S23 x 1017'. The following crosses--'2349 x 1017', 'S23 x 104', 'S23 x 16', '2349 x 118' and '16 x 2336'--showed considerable heterosis effects.

Branch number per plant. In four cross combinations, the F1 plants produced fewer branches than did parental genotypes (Table 2). F1 plants in the following combinations--'1017 x 118', 'Progres x S23', and '1017 x Fiskeby V'--produced considerable (over 200%) heterosis effects.

Pod and seed number per plants, seed yield per plant. In the seed yield components, the cross combination '1017 x 118' produced heterosis effects greater than 300% when characteristics of F1 plants were compared to parental means (Table 2). The next great heterosis effects were found in the '16 x 2336' and 'Progres x S23' cross combinations. In the following cross combinations--'2349 x 1017', 'S23 x 1017', '16 x Fiskeby V' and '118 x 2336'--the F1 plants produced fewer pods and seeds than did better parental genotypes (Table 2). In general, heterosis effects of seed yield followed the trends of number of pods and seeds per plant.

Hundred-seed weight. Moderate heterosis effects were found in the following crosses--'2349 x Fiskeby V', '2349 x 1017', and '118 x 2336' (Table 2). In other cross combinations, heterosis effects were meaningless or seeds were even smaller than those of parental genotypes. The F1 plants in cross '1017 x 104' produced much smaller seeds than did their respective parents.

Discussion: The crossing of well adapted breeding lines selected out in Polish conditions showed that only one combination produced heterosis effects comparable to those reported previously (Konieczny, 1986). However, recorded heterosis effects were considerably greater than those observed by Kunta, 1985. Relatively smaller heterosis effects may be explained by the efficiency and adaptation of breeding material used in crosses. The differences between F1 plants and their parents were relatively small. In spite of good performance of parental genotypes, their F1 plants tended toward a longer vegetation period. It is worth noting that crossing of very late maturing lines resulted in F1 plants with a great reduction in seed yield per plant. This may limit field use of late maturing genotypes with high yield potential

Table 1. Characteristics of parental genotypes in Wielkopolska conditions.

Genotype	Origin	Vegetation period (days)	Plant height (cm)	Branch number /plant	Pod number /plant	Seed number /plant	Seed yield /plant (g)	Hundred seed weight (g)
Fiskeby B	Cultivar Sweden	117.5	46.6	3.4	51.1	98.0	17.6	18.0
Progres	Cultivar Poland	11.62	43.3	2.9	48.0	87.3	15.1	17.2
S23	Fiskeby V	117.1	48.9	4.5	74.8	152.1	21.7	13.7
R18	x PI 180,502							
	Fiskeby V	121.0	51.3	5.4	84.0	131.0	28.4	18.8
	x PI 180,502							
16	Fiskeby V	118.8	56.8	4.7	61.1	105.1	20.2	19.2
	X PI 194,643							
104	"	118.7	43.2	4.5	64.1	119.9	19.4	15.7
118	"	114.0	46.1	3.7	55.3	103.9	16.5	15.7
1017	Fiskeby V	150.0	41.9	6.9	71.1	139.7	22.1	15.6
	x PI 180,499							
2336	PI 194,645	125.5	42.1	4.8	66.2	125.9	21.2	16.8
	x PI 196,491							
2349	PI 196,485	126.9	40.0	3.5	41.9	81.0	14.1	17.4
	x PI 194,645							

Table 2. Mean values of selected characteristics of F1 plants and their heterosis effects as compared with better parent and mean of both parents, 1988.

Cross combination	Number of plants	Vegetation period (days)	Plant height (cm)	Branch number /plant	Pod number /plant	Seed number /plant	Seed yield /plant (g)	Hundred seed weight (g)
Progres x S23								
F1	6	119.3	56.2	6.5	93.3	191.0	26.7	13.9
A (%)		102.9	127.7	216.7	154.3	150.0	152.1	87.9
B (%)		101.1	136.7	232.1	178.8	185.9	178.0	93.9
S23 x 16								
F1	7	116.6	72.4	6.7	79.7	158.0	24.9	15.8
A (%)		104.1	142.5	138.9	122.3	113.0	115.3	87.9
B (%)		102.5	147.2	146.5	122.9	128.1	121.6	103.1
S23 x 2349								
F1	7	120.0	69.6	6.6	93.8	185.6	28.7	153.2
A (%)		107.1	125.2	137.5	143.9	132.8	148.7	84.9
B (%)		104.0	136.5	153.5	163.7	157.4	155.9	99.7
S23 x 104								
F1	9	127.6	72.0	7.0	87.3	172.7	26.4	15.7
A (%)		110.2	131.9	134.6	119.4	119.1	125.1	110.3
B (%)		104.7	150.4	140.0	131.1	133.1	141.2	114.9
S23 x 1017								
F1	3	143.7	114.3	7.0	79.3	136.0	22.3	16.4
A (%)		119.0	206.0	82.3	61.2	54.4	55.0	100.4
B (%)		107.7	212.7	90.3	64.6	56.7	57.6	101.3
2349 x Fiskeby V								
F1	4	136.0	44.0	5.0	75.0	133.0	26.8	21.9
A (%)		116.8	92.1	117.6	140.5	125.2	142.5	121.2
B (%)		116.5	94.1	128.2	147.6	135.4	228.2	126.7
2349 x 118								
F1	5	136.2	57.3	4.8	50.5	114.7	19.6	18.6
A (%)		116.8	137.4	109.8	97.3	119.0	104.5	91.6
B (%)		109.2	141.2	125.8	102.8	125.3	110.1	95.7
2349 x 1017								
F1	5	166.0	74.8	5.2	43.8	64.4	11.5	18.2
A (%)		127.6	143.8	74.2	37.7	28.1	31.2	118.3
B (%)		125.7	153.1	92.5	53.3	40.3	44.6	111.5
16 x Fiskeby V								
F1	4	129.3	53.5	3.8	72.8	123.8	23.5	19.5
A (%)		111.0	102.9	83.3	96.3	92.0	85.6	93.1
B (%)		109.2	113.2	85.0	104.8	99.6	91.6	94.1
16 x 2336								
F1	4	147.0	69.8	7.5	131.8	242.0	38.1	15.7
A (%)		124.5	136.8	173.2	211.1	218.4	178.1	82.6
B (%)		105.9	129.2	184.7	212.9	209.7	198.6	90.9

Table 2. (continued)

Cross combination	Number of plants	Vegetation period	Plant height	Branch number /plant	Pod number /plant	Seed number /plant	Seed yield /plant	Hundred seed weight
		(days)	(cm)				(g)	(g)
16 x 2349								
F1	11	130.5	63.8	5.3	77.1	150.0	23.1	17.0
A (%)		114.5	116.2	147.2	132.7	154.0	130.8	94.3
B (%)		113.7	138.5	166.0	144.2	154.1	141.1	97.3
104 x 16								
F1	5	121.0	46.2	4.6	62.2	93.4	14.6	15.8
A (%)		102.5	103.2	100.0	97.5	77.6	73.11	97.5
B (%)		90.3	107.1	110.3	151.1	125.7	119.4	99.0
1017 x 104								
F1	15	143.4	53.6	7.2	114.0	200.4	30.9	13.8
A (%)		112.9	109.1	93.6	146.2	130.9	127.6	79.9
B (%)		103.5	116.5	109.9	147.4	141.5	132.7	83.7
1017 x 118								
F1	4	133.8	54.6	9.0	170.8	274.3	53.4	14.6
A (%)		117.4	117.1	240.0	266.8	311.9	299.7	95.3
B (%)		101.4	130.2	273.6	335.9	383.2	361.9	95.8
1017 x Fiskeby								
F1	4	137.0	39.0	7.8	86.5	165.6	27.3	16.5
A (%)		117.7	81.6	215.3	117.0	155.9	145.2	85.2
B (%)		101.9	88.5	226.6	119.2	162.2	145.4	88.3
118 x 2336								
F1	9	126.0	43.1	4.6	65.0	114.2	19.9	17.5
A (%)		116.6	94.1	78.4	88.3	84.1	88.7	106.9
B (%)		112.9	96.4	95.6	103.1	97.7	103.5	108.4

F1 - mean value of F1 plants

A (%) - heterosis effect as compared with better parent.

B (%) - heterosis effect as compared with mean of both parents.

as parental components in high latitudes. In the cross combinations with parental lines closely related (e.g., common one parent) the heterosis effect was absent. It seems that in creating crossing programs in high latitudes, the origin and degree of adaptation of parental lines should be considered.

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Jerzy Nawracala

Grzegorz Konieczny

UNIVERSITY OF CUKUROVA

Faculty of Agriculture

Department of Field Crops

Adana 01330 Turkey

TURKEY

1) Effects of some growth regulators on double-cropped soybean production.

Introduction: The soybean planting area is 67,077 ha in the Cukurova region, which has the most productive land in Turkey in 1990 (Anon., 1990). Soybean farming can be done successfully as a second crop after wheat harvest in Cukurova, due to suitable growing period (115-135 days) and environmental conditions. It is very profitable for the farmers, if it is compared with the other production patterns. For this reason, 80-85% of the total production of soybean is the second crop in Turkey.

Soybean has been recognized as quantitative short day plants. Soybean growth and development are significantly affected by environmental conditions. For this reason, the vegetative and generative growth and development of soybean can show important variation, according to photoperiod and temperature, when it is planted as the second crop. Environmental conditions directly affect endogenous plant hormones, and these hormones regulate plant growth and development. These endogenous plant hormones are: gibberellins, auxins, cytokinins, abscisic acid, and ethylene (Gardner et al., 1985). Important variation in growth and development of plants are due to endogenous hormones and their amount. This variation affects seed yield and some important characteristics of the soybean both positively and negatively. The purpose of this study was to determine the influence of GA₃, Atonic, Pix, Cytozyme crop plus and Terpal applications on seed yield and some agronomic characteristics of field-grown soybeans.

Materials and Methods: This study was conducted in Adana in 1988. Adana is located at 37° 19' N latitude in the southern part of Turkey. In this study Amsoy 71 cv. was used. Amsoy 71 was a very common soybean cultivar in Turkey in that year. GA₃, Cytozyme, Atonic, and Terpal were used as plant growth regulators.

A randomized complete block design was used with three replications. The plot size was 5 m long and 2.5 m wide, and each plot has four rows. Seeds were planted at the rate of 80 kg/ha on June 20 as a second crop after wheat harvest. The seeds were inoculated with Rhizobium japonicum and fertilizer

(200 kg/ha DAD) was applied at the time of planting. All plots were irrigated after planting to obtain a uniform emergence of stand. Irrigation was applied by flood irrigation system four times. GA₃ was applied at the rate of 25 ppm at V2 stage, Atonic was 1.5 L/ha (0.5+0.5+0.5 L/ha) at three times in seven days interval, beginning at R7 stage. Cytozyme was 0.9 L/ha (0.45+0.45 L/ha) at two times in 50% and full flowering stages. Pix was 1 L/ha in R7 stage; and Terpal was 1.0 L/ha in R7 stage. These regulators were applied with "knapsack sprayer" using the 3 L/13 m² water.

Plots were harvested by hand and threshed in a stationary plot thresher. Seed was oven dried and yields were adjusted to 13% moisture content. At harvest, plant height, lowest pod height, node number, branch number, harvest index, oil content, pod number, seed number per pod, 100-seed weight and seed yield data were collected, using INTSOY (1976) methods.

Climatical data over the growing period in Adana is given in Table 1.

Table 1. Climatic data over the growing period in Adana, Turkey, in 1988.

Month	Temperature (°C)			RH (%)	10 cm. Soil Temp. (°C)	Total Rainfall (mm)
	Max.	Min.	Average			
May	30.6	11.6	22.3	66.2	24.2	62.9
June	37.7	16.5	25.2	69.5	28.0	60.5
July	41.5	20.1	30.1	57.7	33.3	---
August	38.2	19.0	29.1	64.2	34.2	---
September	38.0	16.5	26.2	57.4	30.3	0.5
October	34.0	10.4	20.3	64.7	21.6	107.1

Source: Meteorological Surveys of Adana in 1988.

The soil type of the experimental area is sandy-loam and soil pH ranged between 7.5 and 7.6 and it has low organic matter and high lime content.

Results and Discussions: Data obtained from the research are given in Tables 2 and 3. As can be seen in table 2, plant height varied between 64.4 and 86.5 cm. Pix and terpal applications reduced plant height due to growth retardant effects. Greatest plant height was obtained from atonic application (86.5 cm). Lowest pod height was increased by GA₃ and atonic application. This is an important plant characteristic for reducing harvest losses.

Pod number per plant was found to be 6.7 N/ plant higher than the check in cytozyme crop plus application. Highest seed number per pod value was obtained from pix and GA₃ application (1.84 and 1.80 N/plant).

Weight of 100 seeds varied between 17.53 and 19.84 gr. The higher 100-

Table 2. Effect of GA₃, Atonic, Cytozyme, Pix and Terpal on plant height, lowest pod height, node number, branch number, pod number, and seed number per pod data for soybeans (1988)

Treatments	Plant height (cm)	Lowest pod height (cm)	Node number (N/P)	Branch number (N/P)	Pod number (N/P)	Seed number (N/P)
GA ₃	83.7	12.3	15.1	2.57	36.2	1.80
Atonic	86.5	10.9	16.0	2.80	35.2	1.65
Cytozyme	82.3	9.5	15.0	2.47	39.0	1.78
Pix	80.3	7.5	14.7	2.27	35.9	1.84
Terpal	64.4	5.1	12.5	2.27	35.9	1.73
Check	84.3	9.5	15.7	2.00	32.3	1.62

CV (5%)	2.93	10.32	5.33	6.45	4.11	5.39
LSD (5%)	4.30	1.70	1.40	6.45	2.60	0.17

seed weight values were found in cytozyme and atonic applications, and the lowest in terpal application (Table 3). Seed yield is a very important characteristic in soybean. It varied between 2461.5 and 2923.1 kg/ha, according to applications. Cytozyme and GA₃ applications have been effective on seed yield. Highest seed yield was obtained from cytozyme application. Seed yield differences were found to be +120.7 kg/ha in Atonic, +179.7 kg/ha in Pix, +254.1 kg/ha in GA₃ and +374.6 kg/ha more in cytozyme applications than the check.

Terpal applications reduced seed yield 87.0 kg/ha less than the check. These findings were in agreement with other investigations (Morandi et al., 1983; Halwanter et al., 1984; Hurduc et al., 1984; Arioglu and Isler, 1989).

In conclusion, some plant characteristics were significantly affected by the GA₃, Atonic, Pix, Terpal and Cytozyme applications. Lowest pod height and seed yield per hectare are the most important characteristics in second crop soybean farming. For these reasons, 25 ppm GA₃ at V2 stage and 90 L/ha Cytozyme crop plus application at 50% and full flowering stages are recommended.

Table 3. Effect of GA₃, Atonic, Cytozyme, Pix, and Terpal on harvest index, 100-seed weight, oil content, seed yield, and yield differences data for soybean (1988).

Treatment	Harvest index (%)	100-seed weight (gr)	Oil content (%)	Seed yield (kg/ha)	Yield differences (kg/ha)
GA ₃	38.58	19.55	20.93	2806.6	+254.1
Atonic	36.93	19.78	21.39	2669.2	+120.7
Cytozyme	39.65	19.84	21.24	2923.1	+374.6
Pix	36.35	17.73	21.30	2728.2	+179.7
Terpal	38.70	17.53	21.02	2461.5	-87.0
Check	40.22	17.78	21.24	2548.5	0.0

CV (5%)	4.82	4.82	1.84	2.77	.-
LSD (5%)	3.37	1.64	NS	135.60	.-

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Halis Arioglu

2) Screening of new soybean varieties for Cukurova ecological conditions as a double crop.

Introduction: Cukurova region, located at 37° 19' N latitude in the south of Turkey, has very productive land. The main crops are cotton and wheat, in this area. Because of suitable climate and soil conditions, it is possible to grow soybeans as a second crop after wheat harvest. Cukurova region has 1.2 million hectares available land and 50% of the available land is irrigated. Double crop soybean farming has been very profitable for the farmers. For this reason, it increased very rapidly and reached 95,000 ha in 1987, while it had been 122 ha in 1980 in the same region (Tarismal Yapi ve Uretim, 1987). More than 1000 soybean cultivars were tested as second crop in this area, cooperating with INTSOY so far. Seed yield per hectare increased to 2.5-4.0 ton from 1.0-1.5 ton. The main reasons for this yield increase are release of new cultivars and improvement of growing techniques. Soybean cultivars belonging to Maturity Groups II, III, and IV are suitable for second crop in Turkey. A lot of soybean cultivars are improved by the breeders every year throughout the world. The purpose of this study was to determine the most suitable soybean cultivars among the new improved ones as a second crop in Cukurova in Turkey.

Materials and Methods: The study was conducted in the experimental area of the University of Cukurova as a double crop in 1989 growing season in Adana, Turkey. Experimental design was a randomized block with three replications. Twenty new cultivars and three standard cultivars (A 3127, AP 240, and Mitchell 410) were screened as a second crop.

Plot sizes were 2.6 x 5.0 m = 13 m² and row spacing was 0.65 m. Soybean seeds were inoculated with Rhizobium japonicum and fertilizer (250 kg/ha DAP) was broadcast at the time of planting. Seeds were planted by hand on June 9. Irrigation was applied by flood irrigation system four times. INTSOY methods were used to obtain data. Pod number, plant height, oil and protein content, whitefly damage and seed yield characteristics were investigated.

The soil type of the experimental area is sandy-loam and soil pH ranged between 7.2 and 7.5. The soil organic matter is low and it contains a high amount of lime.

Table 1. Maturity groups, plant height, lowest pod height, pod number per plant, seed number per pod, and harvest index value data in screened soybean cultivars.

Cultivars	Maturity groups	Plant height (cm)	Lowest pod height (cm)	Pod number per plant (N/P)	Seed number per pod (N/P)	Harvest index (%)
CMS 4982	IV	108.40	15.93	46.66	1.89	24.30
SGI 3306	III	96.36	17.63	55.20	2.17	30.82
Lawrance	IV	94.63	16.63	41.46	1.73	27.10
Mitchell 410	V	93.16	16.96	40.30	2.10	21.43
C 1647	III	86.80	18.10	38.96	2.10	34.92
P 9301	III	85.96	16.23	43.83	2.25	43.17
EX 3626	III	85.26	17.90	38.90	2.13	32.37
J 125	II	84.90	16.53	40.33	2.12	28.20
J 335	III	84.10	14.03	39.03	2.10	29.29
P 9272	II	83.83	18.00	44.83	2.26	33.26
CM 389	III	80.46	14.00	61.43	1.90	29.21
AP 240	II	80.33	15.36	32.56	2.11	39.01
AP 3773	III	80.03	16.40	38.20	2.39	35.99
A 3127	III	79.10	18.30	38.33	2.34	39.46
SGI 3307	III	78.70	15.33	32.83	2.09	30.28
CX 345	III	77.90	15.30	49.76	2.14	35.96
S 2596	II	76.53	11.80	39.80	2.28	46.85
P 9331	III	76.50	13.66	41.36	2.16	39.03
Sherman 3133	III	75.73	14.13	43.43	1.83	35.90
P 9293	II	72.40	14.80	48.83	2.26	32.37
CX 415	IV	68.80	12.06	49.06	1.94	8.23
RHS 6623	III	67.66	15.36	28.33	1.74	15.03
J 396	III	61.80	17.63	14.10	1.62	6.11
LSD (5%)		10.65	3.48	12.73	0.28	6.87

Maximum air temperature increased up to 43.0°C in 1989, but normally it was 28.6 - 43.0° during the growing period. Rainfall was very low during the growing period and for this reason, sufficient water was supplied by irrigation. The relative humidity varied between 57.0% and 70.5% during the growing period.

Results and Discussion: Plant height of the tested soybean cultivars ranged from 61.8 to 108.4 cm. Highest plant yield was obtained from JMS 4982, and the lowest from J-396 cultivar (Table 1). Plant height correlated positively and significantly with seed yield, 100-seed weight, and lowest pod height. The lowest pod height varied between 11.80 and 18.30 cm. Highest value was obtained from A 3127, whereas S 2596 was the lowest. Correlation between lowest pod height and plant height was positive and significant. Highest number of pods per plant was obtained from CM 389 with 61.43 pods/plant, and the lowest from J-396, with 14.10 pods/plant (Table 1.)

The highest seed number per pod was obtained from AP 3773, with 2.39 seeds/pod, and the lowest was found in J-396, with 1.62 (Table 1). Positive and significant correlation was found between seed number/pod and seed yield, 100-seed weight, and harvest index.

Highest 100-seed weight was found in cultivar C 1647, with 18.34 gr, and the lowest was in cultivar J 396, with 8.09 gr. Positive and significant correlation was obtained between 100-seed weight and seed yield, plant height, oil content, and harvest index. The highest oil content was obtained from C 1647, with 21.86%, and the lowest from P 9293, with 17.73% among the cultivars. Oil content positively correlated with 100-seed weight. The average seed yield per hectare ranged between 166.7 and 3615.3 kg. Seed yield was highest in cultivar P 9301. Seed yield of this cultivar was higher than local cultivars. A positive correlation was found between seed yield and harvest index, 100-seed weight, plant height, and seed number per pod. These findings are in agreement with the results of Atakisi, 1978; Atakisi and Arioglu, 1983; Ipkin et al., 1989; Ede, 1986, and Ede and Nazlican, 1989.

In conclusion, the highest seed yield was obtained from P 9301, A 3127, S 2596, and AP 240 cultivars. A 3127 and AP 240 cultivars are used very commonly for double crop soybean production in the Cukurova region. P 9301 and S 2596 were found as new cultivars for the Cukurova region in Turkey. These two new cultivars were recommended to farmers to use as a second crop after wheat harvest.

Table 2. Whitefly damage, seed yield, 100-seed weight, oil content and protein content values in screened soybean cultivars.

Cultivars	Whitefly# damage (1-10)	Seed yield (kg/ha)	100-seed weight (gr)	Oil content (%)	Protein content (%)
S 2596	1	3410.00	15.20	19.46	36.60
P 9301	3	3615.30	13.90	21.00	31.50
A 3127	2	3546.00	14.50	18.06	37.62
P 9331	4	2666.70	11.70	19.60	33.25
AP 240	1	3051.30	13.60	20.93	36.02
AP 3773	4	2358.70	15.12	20.40	34.85
CX 345	5	2359.00	12.55	19.00	38.35
Sherman 3133	5	2192.30	12.10	19.06	38.64
C 1647	4	2474.70	18.34	21.80	34.56
P 9272	3	2794.70	14.50	19.10	30.77
P 9293	1	2694.70	13.35	17.73	36.75
EX 3626	5	2271.70	13.10	20.13	35.73
SGI 3307	5	2589.70	14.53	21.00	38.79
SGI 3306	4	2697.70	12.45	18.66	36.05
J 335	4	2564.30	13.30	19.60	35.44
CM 389	5	1792.30	12.15	20.80	29.46
J 125	3	1446.00	11.10	19.53	36.02
Lawrance	2	1896.30	12.55	20.26	38.35
JMS 4982	5	2256.30	12.70	19.93	36.02
Mitchell 410	6	1385.00	11.82	20.56	40.10
RHS 623	9	546.30	10.70	18.33	34.56
CX 415	10	215.70	8.44	17.93	33.39
J 396	10	166.70	8.09	18.46	43.31

LSD (%)		15.34	10.42	2.37	8.44

#Whitefly damage was given a 1-10 scale (1=resistant; 10=susceptible).

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Mehmet Arslan

Halis Arioglu

UNIVERSITY OF NOTTINGHAM

Plant Genetic Manipulation Group

Department of Botany

Nottingham UNITED KINGDOM

UNITED KINGDOM

1) Regeneration of plants from protoplasts of *Glycine clandestina* Wendl.
accession G1826.

Introduction: In a study by Kollipara and Hymowitz (1989) of the regeneration from leaf explants of various accessions of *G. clandestina*, cultured in accordance with Hymowitz et al. (1986), only six of the 33 accessions tested produced shoots. However, all leaf explants from one of these six accessions, specifically G1826, regenerated. In previous plant regeneration studies of perennial *Glycine* species, we have observed a correlation between the potential for shoot regeneration from complex explants and the development of shoots from protoplast-derived callus. Protoplasts with a high plating efficiency and plant regeneration are of use in genetic manipulations involving techniques such as somatic hybridisation and transformation by direct uptake of DNA. Experiments were undertaken to establish whether the regeneration response demonstrated by leaf explants of accession G1826 was also expressed in protoplast-derived calli.

Materials and Methods: Seeds were obtained from Dr. A.H.D. Brown, CSIRO Division of Plant Industry, PO Box 1600, Canberra, A.C.T. 2601, Australia. Seedling germination, protoplast isolation, and subsequent culture in agarose droplets bathed in liquid media was as described previously (Hammatt et al. 1987). The liquid medium, initially K8P (Gilmour et al., 1987), was replaced weekly with 5 ml volumes of 2:1, 1:1, and 1:2 mixtures of K8P:SC2 (Hammatt et al., 1986) media, and finally by SC2 medium alone. Micro-calli (1-2 mm in diameter) were transferred from agarose droplets to either SC2 or SC6 media (Hammatt et al., 1986) made semi-solid by the addition of 2.5 g/l of Phytigel (Sigma).

Results and Discussion: After 9-10 weeks of culture, micro-calli (1.2 mm diameter) were transferred from agarose droplets to Phytigel-solidified media. Necrosis occurred in 16% of calli cultured on SC6 medium and in 23% cultured on SC2 medium. Of the calli remaining after 2 months of culture on semi-solid media, all produced shoots on SC6 and 35% on SC2. Therefore, 34% of protoplasts cultured at a density of $2.0 \times 10^3/\text{ml}$ with subsequent transfer

to Phytigel-solidified SC6 medium formed tissues capable of shoot regeneration. This is considerably higher than previously reported for G. argyrea G1626 (6.4%; Hammatt et al., 1989) or G. canescens G1171 (1.2%) and G. clandestina G1231 (0.4%; Hammatt et al., 1987). It is interesting that the

Table 1. Percentage of protoplasts undergoing division after 10 days of culture.

	Plating density (protoplasts/ml)					
	5x10 ⁴	2.5x10 ⁴	1x10 ⁴	5x10 ³	2x10 ³	1x10 ³
Liquid culture	77	80	87	89	91	85
Agarose droplets	93	93	93	94	91	88

Table 2. Percentage of protoplasts producing colonies exceeding 1 mm diameter.

	Plating density (protoplasts/ml)					
	5x10 ⁴	2.5x10 ⁴	1x10 ⁴	5x10 ³	2x10 ³	1x10 ³
Liquid culture	Division ceased at 8-cell stage					
Agarose droplets	0.1	1.1	*	*	40	22

* Note. Dense colony growth; impossible to count colonies.

regeneration response from calli plated on SC6 medium, which contains 0.2 mg/l of 6-benzylaminopurine (BAP), was higher than on SC2 with 1.1 mg/l BAP. In previous experiments, SC2 medium was used to induce a regeneration response in protoplast-derived calli of perennial Glycine species (Hammatt et al., 1987; Hammatt et al., 1989). However, the substitution of Phytigel for agar in the medium may have affected the hormonal requirements of protoplast-derived tissues. The use of Phytigel aids the diffusion of phenolic components from cultured tissues, and an increased rate of diffusion may also enhance the effective concentration of media components, including growth regulators. Alternatively, the increased regeneration efficiency at low cytokinin concentrations may have been due to the presence of endogenous cytokinins influencing plant production.

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B. Jones

M. R. Davey

2) Plant regeneration from tissues of Glycine argyrea Tind. transformed by Agrobacterium rhizogenes.

Introduction: The natural infectivity of A. rhizogenes has previously been used to transform wild perennial Glycine species (Rech et al., 1988a, 1988b) with the production of kanamycin-resistant plantlets of G. canescens (Rech et al., 1989). This report describes the regeneration of transformed plants from roots obtained following the inoculation of seedling hypocotyls of G. argyrea with A. rhizogenes wild type strains LBA9402 and A4T, and strains R1601 and A4TIII, which have genetically engineered Ri plasmids. The latter carried the neomycin phosphotransferase (nptII) gene, which confers kanamycin resistance on transformed plant cells. Tissues transformed by A. rhizogenes strains R1601 and A4TIII expressed the nptII gene. Shoot regeneration from roots of G. argyrea G1626 transformed by strain R1601 was found to be

kanamycin-dependent. Additionally, the effects are described of seedling age and bacterial concentration on the transformation response of G. argyrea Gl420.

Materials and Methods: Plant material. Seeds were obtained from Dr. A.H.D. Brown, CSIRO Division of Plant Industry, PO Box 1600, Canberra A.C.T., 2601 Australia. Seeds were scarified before surface sterilization for 15 min in 10% v/v sodium hypochlorite with a few drops of Tween 20, and rinsed 5 times in sterile water. The seed were kept in sterile water on a rotary shaker (150 rpm) at 27°C for 24 h. Seed germination was as described by Rech et al. (1989).

Bacterial cultures. Bacteria were grown on agar-solidified (1.5% w/v; Sigma) APM or YMB media with the addition of antibiotics as appropriate. Cultures for inoculation of seedling hypocotyls were initiated by transfer of single bacterial colonies from agar plates to 10-ml aliquots of liquid medium for 16 hr at 150 rpm (27°C). The 16-hr bacterial cultures with 3.5×10^9 cells/ml (determined by serial dilution plating and spectrophotometry) were diluted with liquid medium to the required concentration (1×10^5 - 1×10^9 cells/ml) for inoculation.

Strain	Plasmid	Maintenance media	Reference
LBA9401 ¹	pRi 1855	YMB	1
A4T ²	Cm ^r Tc ^r Rif ^r derivative of <u>A. tumefaciens</u> C58 cured of pTiC58 carrying pRiA4b.	APM	2
R1601 ³	pRiA4b with a chimaeric <u>nptII</u> gene co-integrated into TL-DNA, and pTK291 in <u>trans</u>	APM with ampicillin and kanamycin both at 50 ug/ml	3
A4TIII ⁴	A4T carrying pRiA4::pAM Neol0	APM with 50 ug/ml of kanamycin	4

¹Ooms et al. (1985). ²Moore et al. (1979). ³Pythoud et al. (1987).

⁴Morgan et al. (1987).

Induction and culture of transformed roots. The preparation, inoculation and subsequent culture of the G. argyrea seedlings was as described by Rech et al. (1989). The bases of inoculated hypocotyls were

inserted into 50 ml of MSO (Murashige and Skoog, 1962) medium contained in 175-ml glass jars, with 5 hypocotyls per jar. Controls were made by the inoculation of hypocotyls with sterile bacterial broth.

Roots produced at the inoculation sites were excised 21-42 days after infection and transferred to 9.0-cm plastic Petri dishes, each containing 20 ml of hormone-free B5 medium (Gamborg et al., 1968; designated B50) containing 3.0% sucrose and 0.9% w/v agar (Sigma), pH 5.8, supplemented with 300 ug/ml of cefotaxime (Claforan; Roussel Laboratories, Uxbridge, U.K.). The root cultures were incubated under continuous daylight fluorescent illumination (0.5 W m^{-2}) at 27°C. Every 14 days, the tips (2-3 cm in length) of the cultured roots were excised and subcultured onto fresh medium. After five passages, the roots were maintained on B50 medium lacking cefotaxime. Kanamycin sulphate (50-100 ug/ml) was also added to medium used for the culture of roots resulting from inoculation with A. rhizogenes strains R1601 and A4TIII.

Shoot regeneration. Root segments (3-4 cm in length) were transferred to agar-solidified SC2 medium (Hammatt et al., 1986) with a 10-day subculture period, for 40-50 days, under continuous daylight fluorescent illumination (1.6 W m^{-2}) at 27°C, to induce callus production and shoot bud formation. Regenerating callus was transferred to agar-solidified SC6 medium (Hammatt et al., 1986). Kanamycin sulphate (50 ug/ml) was added to all cultures resulting from inoculation with A. rhizogenes strains R1601 and A4TIII. However, after the first subculture of callus derived from roots of G. argyrea G1626 transformed by strain R1601, the kanamycin was excluded in an attempt to prevent excessive production of phenolic compounds. After 18 months of culture on antibiotic-free medium, 42 of the G. argyrea G1626 calli were transferred to medium containing 50 ug/ml of kanamycin sulphate. Before transfer to kanamycin-containing medium, transgenic tissues of G. argyrea G1626 were checked for possible bacterial contamination by placing 1-mm thick slices of callus in liquid LB medium (0.5% w/v Difco Bacto yeast extract, 0.5% casaminoacids, 0.8% mannitol, 0.2% w/v $(\text{NH}_4)_2\text{SO}_4$, 0.5% w/v NaCl, pH 7.0) or APM medium (1% w/v Difco Bacto tryptone, 0.5% w/v Difco Bacto yeast extract, 0.5% w/v NaCl, pH 7.0) with shaking at both 27°C and 37°C for 14 days.

Opine analysis, NPTII assay and DNA hybridization. All bacterial strains used encode for agropine, mannopine, and mannopinic acid. Opines were detected by paper electrophoresis (Morgan et al., 1987). NPTII activity in transformed tissues was measured by the method of McDonnell et al. (1987) with

modifications (Tomes et al., 1990). DNA was extracted from Agrobacterium-induced roots, shoot regenerating callus and nontransformed seedlings by the method of Rech et al. (1988a). Extracted DNA (8 ug) in 20 ul of extraction buffer (200 mM Tris HCl pH 7.5, 250 mM EDTA pH 8.0 and 0.5% w/v SDS) was denatured by heating to 95°C (5 min) followed by chilling on ice (2 min). Aliquots of 2 ul of each sample were spotted onto nylon Hybond-N membrane (Amersham) and air dried. Membrane was wetted in a denaturing solution (0.5 M NaOH and 1.5 M NaCl) for 1 min, followed by air drying. The DNA was fixed, hybridized with labelled probe and washed (Morgan et al., 1987). The pFW94 probe DNA for pRi TL-DNA (Huffmann et al., 1984) was ³²P-labelled using a nick translation kit (BRL). Filters were exposed to X-ray film (Kodak, X-Omat S) with an intensifying screen (Cronex Quanta III) at -70°C for up to 9 days.

Results and Discussion: G. argyrea G1420. The frequency of root production at inoculation sites, 42 days after infection of G. argyrea G1420 hypocotyls, influenced by both seedling age and bacterial concentration of the inoculum. Of 9- to 27-day-old seedlings infected with an inoculum of 1.0×10^8 bacteria/ml, 9-day-old seedlings were most responsive to infection by strains LBA9402, R1601, and A4TIII, giving transformation responses of 35%, 34%, and 22%, respectively. However, hypocotyls from 14-day-old seedlings gave the highest response (36%) to inoculation with A4T. Of the concentrations of bacteria used to infect hypocotyls of 9-day-old seedlings, a suspension of 1.0×10^7 bacteria/ml gave the optimum transformation response (37%), whereas 1.0×10^8 bacteria/ml was more effective with strains A4T, R1601, and A4TII.

Within 20 days of the transfer of transformed roots to SC2 medium, root growth was inhibited and hard, green, nodular callus was formed. This callus developed shoot buds after 40-50 days, which elongated following transfer to SC6 medium. Excised shoots developed extensive root systems when transferred to B50 medium lacking growth regulators.

All of the tissues derived from roots transformed with strains LBA9402, A4T, R1601, and A4TIII contained silver nitrate positive compounds that migrated to the same positions on electrophoresis as agropine, mannopine, and mannopinic acid standards. These compounds were not detected in callus initiated from nontransformed roots. Hybridization of the ³²P-labelled probe pFW94 carrying Ri TL-DNA to total DNA isolated from shoots transformed by strains LBA9402, A4T, R1601, and A4TIII, but not to DNA from nontransformed seedlings, confirmed stable Ri T-DNA integration into the genome of G.

argyrea.

Roots, calli, and regenerated shoots derived from roots transformed with the engineered strains R1601 and A4TIII were the only tissues that continued to grow on B50 medium containing 50-100 ug/ml of kanamycin sulphate. The expression of the nptII gene in these tissues was confirmed by specific NPTII enzyme activity, which was 20 to 28 times greater than in nontransformed tissues.

G. argyrea Gl626. Thirty days after infection of the hypocotyls of 12-day-old seedlings of G. argyrea Gl626 with a 16-hour culture of A. rhizogenes R1601, 52% of the hypocotyls had produced roots at the inoculation sites. Roots were not produced in the controls. Calli from R1601-transformed roots failed to regenerate response during 18 months of subculture on kanamycin-free SC6 medium. However, when 42 calli were transferred to SC6 medium containing 50 ug/l of kanamycin sulphate, 35 produced shoot buds within 14 days and all had responded by 26 days. During this time, there was still no regeneration response in cultures on kanamycin-free medium. However, the remaining calli all produced shoot buds when they were also transferred to shoot regeneration medium containing 50 ug/ml of kanamycin sulphate.

Plants recovered from R1601-transformed shoots exhibited similar morphological traits to those previously regenerated from transformed roots of G. canescens and G. clandestina (Rech et al., 1988a). Transformed roots of G. argyrea 1626 expressed a specific NPTII activity of 73.65 ± 1.56 , compared with 0.73 ± 0.02 for roots of nontransformed seedlings; regenerated shoots had a level of 8.63 ± 0.27 , compared with 0.91 ± 0.03 for nontransformed shoots.

Initially, it was thought that endogenous bacterial contamination may have inhibited shoot regeneration, and that the addition of kanamycin to the shoot regeneration medium was controlling the infection. However, bacterial growth was not observed when 1-mm slices of transformed calli were placed in LB or APM medium for 14 days. There is no obvious explanation for the observed kanamycin-dependent shoot regeneration in terms of gene integration and expression. In theory, the introduction of a foreign promoter sequence into a host genome could affect the expression of the genes neighboring the point of insertion. However, the effect is kanamycin dependent, and as the integrated nptII gene is under the control of the promoter and poly-A signal of the eukaryotic nopaline synthase (nos) gene, regulatory gene control can be ruled out. The addition of antibiotics to culture medium has been reported to

stimulate embryogenesis and regeneration in wheat (Triticum aestivum; Mathias and Boyd, 1986) and barley (Hordeum vulgare; Mathias and Mukasa, 1987). Additionally, Mukherji and Biswas (1985) found that indoleacetic acid (IAA) oxidase activity of rice seedlings was enhanced by penicillin. However, the kanamycin-independent regeneration of shoots has been achieved from calli derived from nontransformed protoplasts (Hammatt et al., 1989), transformed tissues of G. argyrea produced by uptake of plasmid carrying the nptII gene (Jones and Davey, 1991), and roots of G. argyrea Gl420 transformed by wild type strains of A. rhizogenes (Rech et al., 1988a, 1988b; Kumar et al., 1991).

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B. Jones

V. Kumar

N. Hammatt

M. R. Davey

3) Direct gene uptake and regeneration of transgenic shoots from protoplasts of *Glycine argyrea* Tind.

Introduction: In previous experiments by Rech et al. (1989), the neomycin phosphotransferase (nptII) gene was found to confer kanamycin resistance on plants of *Glycine canescens* transformed by *Agrobacterium rhizogenes* strain R1601. The production of transgenic seeds of wild perennial *Glycine* species should expand our knowledge of gene inheritance and expression in legumes, and provide marked lines that can be used in somatic hybridization (Jones et al., 1989). Antibiotic resistance has already been employed as a selectable marker in the production of somatic hybrids between the forage legumes *Lotus corniculatus* and *L. tenuis* (Aziz et al., 1990).

The establishment of transformed plants in a glasshouse is a prerequisite for transgenic seed production. *G. argyrea* G1626 was selected for use in transformation and somatic hybridization studies because of its ability to produce seeds in the U.K., and its high regeneration response from protoplast-derived tissues (Hammatt et al., 1989). Kanamycin-resistant plants of *G. argyrea*, transformed by *A. rhizogenes* R1601 carrying a cointegrate Ri plasmid vector (Pythoud et al., 1987) have been reported (Jones et al. 1991; Kumar et al., 1991). However, the Ri vector also confers a neoplastic phenotype, normally associated with transformed root formation (Moore et al., 1979; Rech et al., 1988), which prevents Ri-transformed plants of *G. argyrea* from developing roots systems following transfer to compost. This problem has been circumvented by regenerating phenotypically normal plants following direct gene uptake into protoplasts. The plasmid pCaMVNEO (Fromm et al., 1986) which encodes kanamycin resistance in transformed plant cells, was introduced into *G. argyrea* G1626 protoplasts using a noncommercial electroporator (Jones et al., 1991). The optimal parameters for electroporation were previously determined from transient gene expression experiments (Jones et al., 1991) involving chloramphenicol acetyl transferase (CAT) gene expression following electroporation of protoplasts in the presence of pDW2.

Materials and Methods: Plant material. Seeds were obtained from Dr. A.H.D. Brown, CSIRO Division of Plant Industry, PO Box 1600, Canberra A.C.T. 2601, Australia.

Electroporation. Seeds of *G. argyrea* G1626 were germinated at 27°C under 1.6 W m⁻² continuous fluorescent illumination. Protoplasts were isolated

from cotyledons (approximately 40 cotyledons per isolation) of 14-day-old seedlings as described by Hammatt et al. (1989), but with two final washes by resuspension in electroporation solution (designated EP9M). The latter consisted of 9% w/v mannitol, 0.5 mM CaCl_2 , and 0.5 mM MES. Protoplast density was adjusted with EP9M solution to $1.0 \times 10^6/\text{ml}$, and 1-ml aliquots transferred to the central wells of 10 cm x 10 cm 25-well Sterilin dishes. Immediately before electroporation, 20 μg of pCaMVNEA were added to each protoplast sample. Two square DC pulses of 750 V/cm for 2 μs were applied to each sample using a parallel plate electrode (sterilized by immersion in 70% v/v ethanol for 10 min before use), based on the design by Watts and King (1984), and a noncommercial pulse generator. Control samples were treated in a similar way, but without plasmid.

Culture of electroporated protoplasts. Electroporated protoplasts were transferred to sterile Eppendorf tubes, each containing 1 ml of K8P liquid culture medium prepared to the formulation of the protoplast culture medium of Kao (1977) with modifications (Gilmour et al., 1989). When the protoplasts had settled to the bottom of the tubes (2-3 hr), the supernatant was removed and the protoplasts resuspended in K8P medium to a density of $1 \times 10^4/\text{ml}$. After incubation in the dark for 16 hr at 27°C, the protoplasts were suspended, at $4 \times 10^3/\text{ml}$ in K8P medium containing 6 g/l of Sigma Type VII agarose. Protoplasts were dispensed as 55 μl droplets in 9-cm Petri dishes to a total of 3 ml per dish, and 10 ml of liquid K8P was added. The protoplasts were cultured as described by Hammatt et al. (1989), but with kanamycin sulphate in the replacement liquid media at concentrations, in each of three separate sets of cultures, of 25, 50, or 100 $\mu\text{g}/\text{ml}$. Protoplast-derived colonies of 1-2 mm in diameter were transferred to SC6 medium (Hammatt et al., 1986) solidified with 2.5 g/l Phytigel. In most of these cultures, kanamycin sulphate at 100 $\mu\text{g}/\text{ml}$ was added to the medium. However, some colonies were maintained on SC6 medium without this antibiotic.

NPTII assay. Transformed shoots, regenerated from cells cultured in K8P medium containing 50 $\mu\text{g}/\text{ml}$ of kanamycin sulphate, were assayed for NPTII activity, using the method of McDonnell et al (1987) with modifications (Tomes et al., 1990).

Results and Discussion: The highest yield of 1-2-mm diameter protoplast-derived colonies was on K8P medium containing 25 $\mu\text{g}/\text{ml}$ of kanamycin sulphate (Table 1), but 49% of these were lost after transfer to SC6 medium

with 100 ug/ml of kanamycin sulphate. However, 79% of the protoplast-derived colonies survived transfer from an initial kanamycin concentration of 50 ug/ml, to SC6 medium, with 100 ug/ml of the antibiotic. Shoot regeneration, from protoplast-derived calli initially cultured on K8P medium containing 50 and 100 ug/ml of kanamycin sulphate, was higher than previously reported for this accession (Hammatt et al., 1989). This may have been related to the replacement of agar with Phytigel (Sigma) as the gelling agent in the SC6 medium (Jones et al., 1991), rather than to the transformation process itself.

Table 1. The effect of kanamycin sulphate on the number of electroporated protoplasts developing into transgenic, shoot-producing calli.

	Kanamycin sulphate concentration		
	25 ug/ml	50 ug/ml	100 ug/ml
No. of 1-2-mm diameter colonies per 1.2 x 10 ⁴ protoplasts	37	19	11
Percent of tissues producing shoot buds	24.3	78.9	54.5
Transformation frequency (protoplasts giving transformed shoots)	75x10 ⁻⁴	1.3x10 ⁻³	9.2x10 ⁻⁴

The specific NPTII activity (number of scintillation counts per min per ug of total protein) was 28.67±2.26 c.p.m. for shoots regenerated from transformed callus and 0.91±0.03 c.p.m. for nontransformed shoots. These values confirmed nptII gene expression in transformed tissues.

In previous experiment involving G. argyrea G1626, callus obtained from roots transformed by A. rhizogenes was found to be dependent on the presence of kanamycin sulphate in the SC6 medium used for shoot regeneration (Jones et al., 1991). However, shoot regeneration from calli obtained by direct gene uptake did not require the presence of the antibiotic in the culture medium to stimulate shoot production.

Currently, transformed shoots are being cultured in root-inducing medium [1.2 strength B5 salts (Gamborg et al., 1968), 1 g/l sucrose, 1 ug/l indole-3-acetic acid and 2.5 g/l Phytigel], and plantlets transferred to potting compost prior to growing to maturity in the glasshouse for seed production. G. argyrea G1626 has proven to be readily transformed by A. rhizogenes (Jones et al., 1991; Kumar et al., 1991) and by direct gene uptake into protoplasts. Future studies will clarify the fertility of transgenic plants resulting from the present experiments.

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B. Jones

M. R. Davey

4) The use of flow cytometry to compare nuclear DNA levels in putative somatic hybrid shoots of *Glycine canescens* G1171 X *G. argyrea* G1626.

Introduction: Techniques exist for the selection of heterokaryons following protoplast fusion (Patnaik et al., 1985; Gilmour et al., 1989; Medgyesy et al., 1985; Pirrie and Power, 1986; Saxena and King, 1987; deVries et al., 1987; Harmes, 1982; Terada et al., 1987; Ochatt et al., 1989; Gilmour et al., 1987; Jones et al., 1990) but despite the efficiency of any particular selection strategy, the hybridity of regenerated tissues must be confirmed by molecular analysis. Previously, we have described the use of flow cytometry to sort heterokaryons from homokaryons and unfused parental protoplasts following the elctrofusion of fluorescently labelled Glycine protoplasts (Jones et al., 1990). Flow cytometry has also been used to measure variation in DNA content of accessions of perennial Glycine species (Hammatt et al., 1990; Blackhall et al., 1991), and to correlate nuclear DNA content with chromosome number in somatic hybrids in the Brassicaceae (Fahleson et al., 1988). The present report describes the use of flow cytometry to obtain relative values for the DNA content of nuclei isolated from parental and putative somatic hybrid shoots. This data can be used to confirm whether a putative hybrid, having a DNA content greater than $2n$, has resulted from the fusion of two or more protoplasts. However, as increases in DNA content can also result from homofusion or changes in the ploidy level, a DNA content greater than $2n$ is itself no confirmation of hybridity. Nevertheless, the advantage of this technique is that many protoclonal lines can be screened very rapidly, and lines appearing to have originated from nonfused (parental) protoplasts (having a DNA content of $2n$) can be eliminated from the investigation prior to molecular analysis.

Materials and Methods: Plant material. Seeds were obtained from Dr. A.H.D. Brown, CSIRO Division of Plant Industry, PO Box 1600, Canberra, A.C.T. 2691, Australia.

Electrofusion. Protoplasts were isolated from cotyledons of light-grown seedlings of *G. canescens* G1171 as described by Hammatt et al. (1986). Protoplasts were isolated from hypocotyls of dark-grown seedlings of *G. argyrea* G1626, by the same procedure, but with the incubation of 1-mm

hypocotyl sections in an enzyme mixture consisting of 1.5 g of Rhozyme (Rohm and Hass Co., Philadelphia), 0.5 g of Cellulase RS (Yakult Honsha Co. Ltd., Nishinomiya Hyogo, Japan), 0.1 g of Pectolyase Y23 (Seishin Pharmaceutical Co., Tokyo) and 5.0 mM MES dissolved in a solution containing CPW salts (Frearson et al., 1973) with 9% (w/v) mannitol at pH 5.8 (CPW9M). Hypocotyl protoplasts were labelled with the fluorescent molecule fluorescein, by the addition of fluorescein diacetate (FDA; 1 ug/ml) to the enzyme solution. Protoplasts were washed twice in electrofusion solution (9% w/v mannitol, 0.25 mM calcium chloride) before resuspension at a density of 5×10^4 /ml in the same solution. The electrical fusion of protoplasts was performed using a noncommercial instrument (Jones et al., 1991). An alignment field of 150 V/cm at 1 MHz, followed by a single 250 us, 600 V/cm DC pulse, was applied to 1-ml aliquots of the two protoplast suspensions, mixed before use in a 1:1 (v:v) ratio.

Heterokaryon selection. When examined with blue light, using a Nikon Diaphot TMD inverted microscope with an IF 420-485 filter, the chlorophyll-containing cotyledon protoplasts fluoresced red, whereas the fluorescein-labelled hypocotyl protoplasts appeared green. Bifluorescent heterokaryons were identified, and collected by micromanipulation (Gilmour et al., 1987).

Heterokaryon culture. The heterokaryons were cultured in 96-well microtitre plates. Each well contained approximately 200 heterokaryons in 200 ul of K8P liquid medium (Gilmour et al., 1987). After one week in the dark at $27 \pm 2^\circ\text{C}$, the protoplasts were plated in 200 ul aliquots of agarose- (Sigma Type VI) solidified K8P medium as thin layers in 24-well plates. The agarose medium was overlaid with 300 ul aliquots of liquid K8P:SC2 medium (3:1, v:v; Hammatt, et al., 1987), and placed under continuous daylight fluorescent illumination of 1.6 Wm^{-2} at $27 \pm 2^\circ\text{C}$. The liquid medium was progressively replaced with 2:1, 1:1 and 1:2 (v/v) mixtures of K8P:SC2 media at seven-day intervals. Micro-calli (1-2-mm in diameter) were transferred to agarose-solidified SC2 medium and subcultured to fresh medium every two weeks. The resulting morphogenic calli was transferred to SC6 medium (Hammatt et al., 1987) for shoot elongation.

Flow cytometric analysis. Shoots were removed from putative hybrid tissues, and parental shoots were obtained from seedlings germinated in vitro (Hammatt et al., 1986). Nuclei were isolated by chopping the shoots in citric acid buffer (21 g/l citric acid with 5 g/l Tween 20). The resultant suspension

was sieved through a 15- μ m nylon mesh, and centrifuged at 100 x g for 10 minutes. The pellet was resuspended in 200 μ l of citric acid buffer, before addition of 1 ml of DAPI solution (1.75 μ g/ml DAPI in 71 g/l $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$). Isolated nuclei were analysed by flow cytometry as described by Blackhall et al. (1991).

Results and Discussion: The relative nuclear DNA contents of the two parental species, G. argyrea G1626 and G. canescens G1171, were found to be similar, as demonstrated by the close positions of their DNA peaks (Fig. 1, A, B). The putative hybrids were identified as those samples with displaced DNA peaks (Fig. 1 C). Hammatt et al. (1990) determined the DNA content per 4C nucleus for G. argyrea G1626 and G. canescens G1171 to be 5.16 pg and 4.83 pg, respectively. The same workers also showed that the nuclear DNA content of naturally occurring allotetraploids of G. tabacina and G. tomentella approximate to the sums of the diploid genomes from which they are thought to originate. However, the relative nuclear DNA content in tissues derived from the fusion of G. argyrea G1626 with G. canescens G1171 was not a simple multiple of parental values, but had a wide range of values (Fig. 2). The flow cytometric analysis of somatic hybrid tissues of G. max cv. HP20-20 with G. canescens G1171 by Hammatt et al. (1988) also showed a 10% reduction in the expected nuclear DNA content. Therefore, some genomic material is lost, either during mitotic events immediately following protoplast fusion and/or by gradual elimination during subsequent culture. Periodic determinations will be conducted on the nuclear DNA levels of the lines examined, to determine whether progressive genomic loss is occurring, as information on the stability of these putative asymmetric Glycine polyploids should be of interest to workers involved in partial genome transfer.

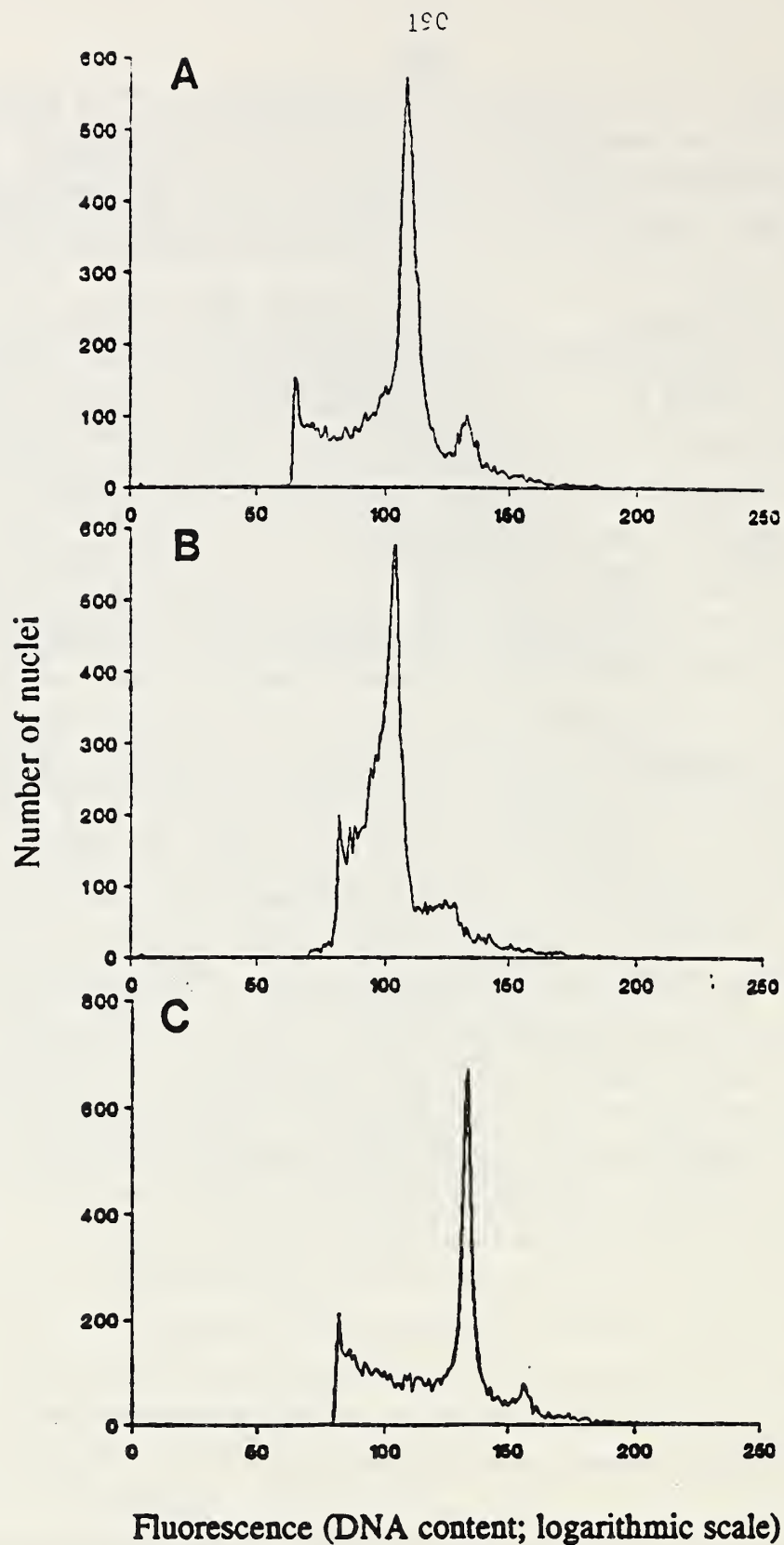


Figure 1. Fluorescence histograms showing the positions of the DNA peaks for *G. argyrea* G1626 (A), *G. canescens* G1171 (B), and a putative somatic hybrid (C).

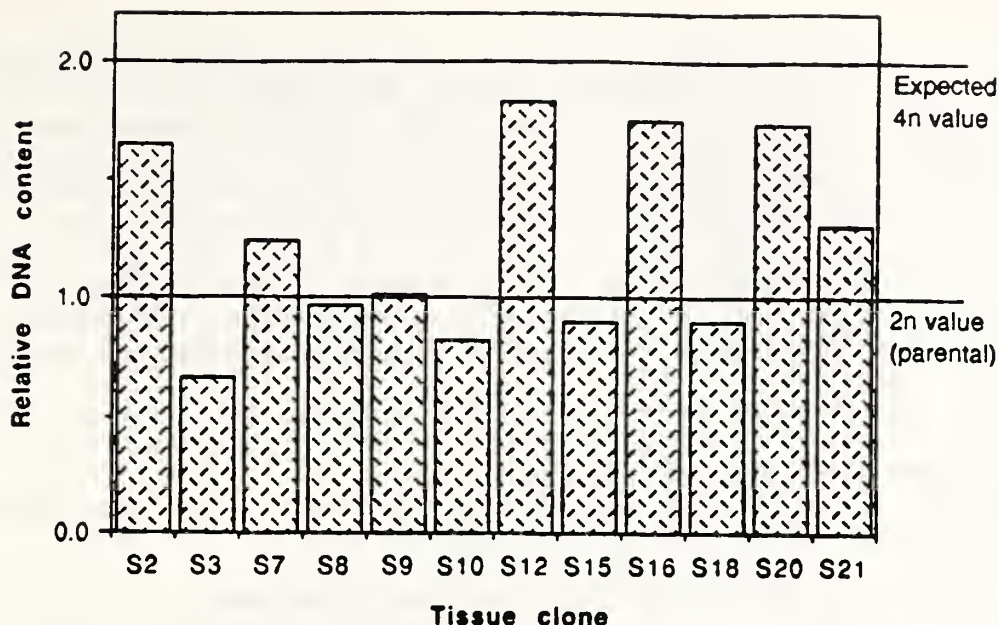


Figure 2. Relative DNA levels of nuclei isolated from shoots regenerated from the fusion of protoplasts of *G. argyrea* G1626 with protoplasts of *G. canescens* G1171.

Some of the clones shown in Figure 2, especially S3, have relative DNA values of less than $2n$. Although this could be experimental error, this is unlikely as clone S3 exhibits a highly abnormal morphology *in vitro*. This morphology, which includes leaf miniaturization, short internode distances and multiple shoot formation, is unlikely to be a direct result of culture. Shoots regenerated from nonfused protoplasts of *G. clandestina* G1826 (Jones et al., 1991) were also examined by flow cytometry, but no significant differences were found in the relative nuclear DNA content of the protoclonal clones examined.

In conclusion, flow cytometry can be used to identify putative somatic hybrids, and asymmetric polyploids.

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B. Jones

N. W. Blackhall

N. Hammatt

M. R. Davey

5) Analysis of variation in the DNA content of Glycine species: A flow cytometric study.

Introduction: Current understanding of the systematic relationships between perennial Glycine species has been derived from morphological and biochemical evidence, as well as studies of meiotic chromosome pairing in interspecific hybrids (Newell and Hymowitz, 1983; Grant et al., 1984; Singh and Hymowitz, 1985 a, b, and Singh et al., 1988). Based on this information, Hymowitz and Singh (1988) have assigned genome symbols to Glycine species. The genus Glycine consists of two subgenera, Soja and Glycine. Soja contains the annual cultivated soybean, G. max L. Merr., and its conspecific, twining progenitor, G. soja. Both G. max and G. soja, which are probably diploids ($2n=2x=40$), have been given the genome symbol GG. The Glycine subgenus contains 12 perennial species, amongst which two important diploid ($2n=2x=40$) genomes have been identified. The A genome comprises G. canescens (AA), G. argyrea (A₁A₁) and G. clandestina (A₂A₂), while the B genome comprises G. microphylla (BB), G. latifolia (B₁B₁) and G. tabacina (B₂B₂). The remaining diploid species have been given genome symbols as indicated in Table 1. G. falcata (FF) demonstrates very low levels of pairing between meiotic chromosomes, leading to the conclusion that it is the most distantly related of the Glycine species. The genomes of G. latrobeana and the newly described species, G. arenaria and G. curvata, have yet to be determined, although some studies of chromosome pairing in interspecific hybrids have revealed some affinity of G. latrobeana to the AA genome (Grant et al., 1984). Seedlings of the newly described diploid species, G. albicans, G. hirticaulis and G. lactovirens were not available to this study. A separate group of allotetraploid species has been identified which is composed of G. tabacina ($2n=80$), together with euploid ($2n=80$) and aneuploid ($2n=78$) G. tomentella. These three species are thought to be allotetraploid complexes (Singh et al., 1987) and, consequently have been designated AAB₂B₂ or BBB₂B₂, AADD and DDEE, respectively.

The present study was established to investigate the range of DNA content in the genus Glycine using flow cytometry. Several laboratories have reported on this technique (Galbraith et al., 1983; Fahleson et al., 1988) and the ability of flow cytometry to resolve intraspecific variation in DNA amounts has been confirmed by Rayburn et al. (1989). In the present report, the 4c DNA content for each genotype was determined by comparison with Allium

cepa, for which the DNA content per 4c nucleus has previously been reported (Bennett and Smith, 1976).

Materials and Methods: Seed sources. Seeds of Glycine accessions with a "G" prefix were obtained from Dr. A.H.D. Brown (CSIRO Division of Plant Industry, PO Box 1600, Canberra, ACT, Australia). Those with a "PI" prefix were donated by C.A. Newell (Monsanto Co., 700 Chesterfield Village Parkway, St. Louis MO 63198), and T. Hymowitz (Department of Agronomy, University of Illinois, Urbana IL 61801), who also provided seeds of accessions with a three-figure number. Seeds of G. soja 3080 were supplied by AVRDC (PO Box 42, Shanhua, Taiwan) and soybeans by W. Ellingson (AgriPro, Ames IA). Seeds of Allium cepa cv. Ailsa Craig were purchased locally (Stewarts Garden Supplies, George Street, Nottingham).

Germination of Seedlings. Seeds of all perennial species were surface-sterilized in 10% v/v "Domestos" bleach solution (Lever Brothers, Ltd., Kingston-on-Thames, U.K.) and rinsed in sterile water. A piece of the testa was removed from each seed. The seeds were soaked in sterile water and placed on the surface of agar-solidified half-strength hormone-free B5 medium (Gamborg et al., 1968) in glass jars. Soybeans and seeds of G. soja were surface-sterilized and rinsed as described above, but the seed were not soaked in water prior to sowing. Glycine seeds were germinated by placing the jars at 27°C under daylight fluorescent tubes. Seeds of Allium cepa were sown on filter paper circles moistened with tap water and germinated in the dark.

Flow Cytometry. Flow cytometry was used to provide a rapid procedure for the assessment of nuclear DNA content. Nuclei were isolated mechanically from seedlings and stained with the quantitative nucleic acid-specific fluorescent dye ethidium bromide, before analysis using a Coulter EPICS V instrument. Approximately 1 g of leaf tissue of each sample was chopped finely in a buffer that consisted of 45 mM MgCl₂, 30 mM sodium citrate, 20 mM MOPS, 10 g/l Triton X-100 and 0.1 mg/ml ethidium bromide. For each sample the instrument measured the fluorescence emitted by the released nuclei, as well as the fluorescence from two types of polystyrene beads which were added as control particles (Fig. 1).

Results and Discussion: For the A genome accessions, the 4c DNA amounts ranged from 3.80 to 5.16 pg. In all four experiments, the relative fluorescence of G. clandestina (A₂A₂; mean 4.88 ± 0.08 pg) was always more than for G. canescense (AA; mean 4.20 ± 0.33 pg). When G. argyrea (A₁A₁) was

compared with the AA and A₂A₂ genomes, the three accessions studied (G1626, 1G2004, and G2010) emitted greater fluorescence than any accession with an AA or A₂A₂ genome. These relationships are summarized (Figure 2).

Amongst the diploid species, two groups could be recognized based on their DNA amounts. The first were those with the A, B, DD, EE, and GG genomes, whose range was 3.80 to 5.29 pg of DNA. The second group of diploids (Figure 2) consisted of G. curvata, G. cyrtoloba (CC) and G. falcata (FF) with DNA amounts of 5.27 to 6.59 pg. The FF genome, with 6.24 to 6.59 pg of DNA, was the most unusual. Interestingly, this genome shows little homology with other Glycine genomes (Singh et al., 1988) and the exceptional nature of G. falcata compared to other diploid Glycine species can also be seen in a range of morphological and biochemical characteristics. The unusual DNA amounts in G. falcata substantiate the view that this genome is distantly related to the genomes of other species. An increase in DNA content may have contributed to the low levels of pairing observed between FF and other Glycine genomes (Singh et al., 1988), which, in turn, may have resulted in the isolation and definition of this species.

Similarly, the low degree of homology between the CC genome of G. cyrtoloba and other genomes suggest a high degree of phylogenetic isolation. This may be expressed and/or affected by the considerable difference in DNA amounts between CC and other genomes. Since very little is currently known about the recently described species, G. curvata, conclusions cannot be drawn from its relative DNA amount.

The polyploid species G. tabacina (2n=80; AABB, BBB₁B₁) and G. tomentella (2n=78, 2n=80; AAEE and DDEE, respectively) contained DNA amounts approximating to the sums of the respective parental diploid species thought to have given rise to these allotetraploids.

This study has shown that intra- and inter-specific differences in the DNA amount of Glycine species are detectable by flow cytometry. The process was facilitated by the small size of the genus with only 17 species and an increasing understanding of Glycine systematics.

Table 1. Flow cytometric estimations of the DNA content of Glycine species.

Species	Genome	Accession	Origin	Number of independent measurements	DNA content (pg per 4c nucleus)
<u>G. canescens</u>	AAA	G1114	NSW	4	3.80
		G1171	NSW	3	4.83
		G1240	NSW	4	4.06
		G1249	NSW	4	4.52
		G1301	NT	3	3.99
		G1340	NT	4	4.18
		G1699		4	4.00
<u>G. argyrea</u>	A ₁ A ₁	G1626	Q	3	5.16
		G2004	Q	2	5.05
		G2010	Q	3	5.06
<u>G. clandestina</u>	A ₂ A ₂	G1003	NSW	4	4.91
		G1019	ACT	4	4.98
		G1145	NSW	3	4.76
		G1201	NSW	4	4.83
		G1231	NSW	4	4.94
<u>G. microphylla</u>	BB	307	NSW	3	3.91
		312	NSW	4	3.99
<u>G. latifolia</u>	B ₁ B ₁	G1137	Q	3	4.31
		G1233	NSW	4	4.27
		G1343	Q	3	4.24
		G1426	NSW	3	4.28
		G1456	Q	3	4.31
<u>G. tabacina</u> 2n=40	B ₂ B ₂	PI 373,986	NSW	3	4.02
		G1138	NSW	4	4.18
		G1195	NSW	3	4.17
		G1206	NSW	3	4.15
		G1317	NSW	4	4.46
<u>G. cyrtoloba</u>	CC	317	NSW	2	5.27
		480	Q	2	6.25
<u>G. tomentella</u> 2n=40	DD	G1300	Q	4	4.12
		G1381	PNG	4	4.51
<u>G. tomentella</u> 2n=38	EE	PI 137,987	NSW	3	4.33
<u>G. falcata</u>	FF	G1246	Q	3	6.24
		G2082	Q	2	6.35
		G2086	Q	2	6.59

Table 1. (continued)

Species	Genome	Accession	Origin	Number of independent measurements	DNA content (pg per 4c nucleus)
<u>G. max</u>	GG	'Essex'	USA	2	4.70
<u>G. soja</u>	GG	3080	T	2	4.56
<u>G. tabacina</u>	AAB ₂ B ₂ /	G1080	NSW	1	8.11
2n=80	BBB ₂ B ₂	G1255	NSW	1	8.96
		G1258	NC	1	8.10
<u>G. tomentella</u>	DDEE PI	373,988	NSW	3	9.03
2n=78					
<u>G. tomentella</u>	AADD	G1134	Q	1	8.54
2n=80		G1146	Q	3	8.38
<u>G. arenaria</u>	-	G1305	WA	3	4.81
		G1931	WA	3	4.64
<u>G. curvata</u>	-	G 1396	Q	2	5.98
<u>G. latrobeana</u>	-	G1251	V	2	5.40
		G1387	V	3	5.17

ACT, Australian Capital Territory; NC, New Caledonia; NSW, New South Wales; NT, Northern Territory; PNG, Papua New Guinea; Q, Queensland; T, Taiwan; V, Victoria; WA, Western Australia.

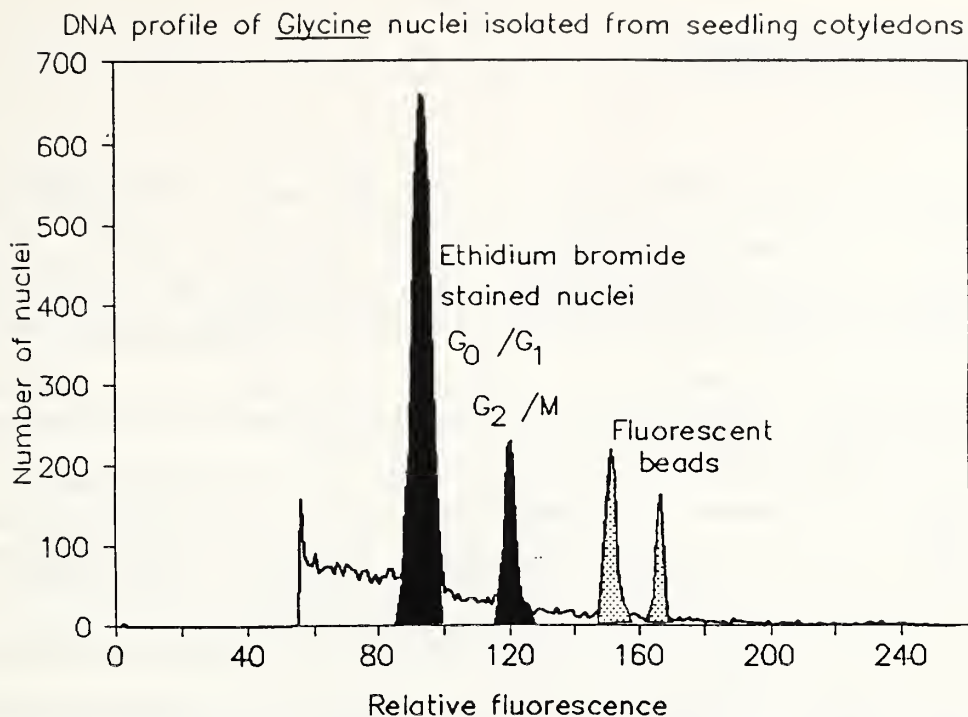


Figure 1. Histogram showing the distribution of log integral fluorescence for G_0/G_1 and G_2/M nuclei isolated from cotyledons of *G. argyrea* G2004, with polystyrene fluorescent beads used as nonbiological internal standards.

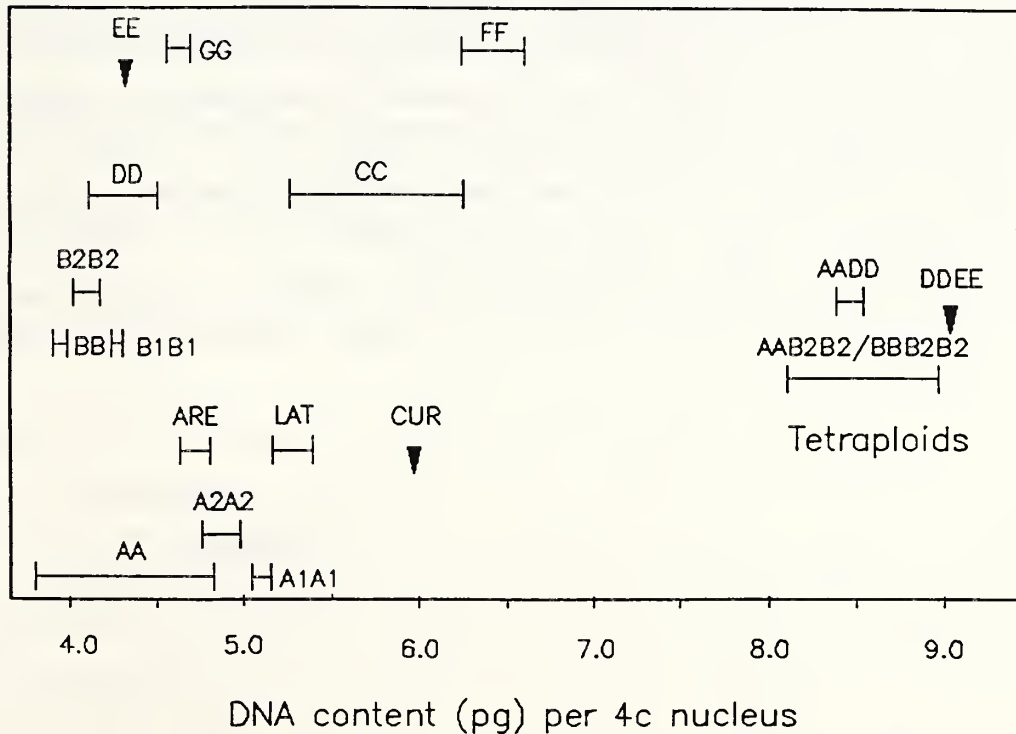


Figure 2. Variation in DNA amounts found in each genome of the genus *Glycine*. ARE, *G. arenaria*; CUR, *G. curvata*; LAT, *G. latrobeana*.

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N. W. Blackhall

N. Hammatt

M. R. Davey

UNITED STATES

AGRACETUS, INC.
8520 University Green
Middleton WI 53562

1) From crown gall to field testing: Historical review of soybean transformation.

The development of an efficient method for the genetic transformation of soybean has been one of the early targets of plant genetic engineering. The dual utility of soybean seed, both as a protein source and a source of oil contributes to the immense value of recovering soybean germplasm with altered genotypes. Desirable agronomic traits for soybean are similar to those of other field crops. Such traits include disease, herbicide and insect resistance, and climatic-stress tolerance. These improvements are directed towards increased yield by allowing the crop to more closely approach its genetic potential in real field environments, and also decreased production costs by reducing the need or expense of chemical inputs where feasible. In addition, improved nutritional and food-processing qualities are important non-agronomic targets due to the widespread use of soybean in food and feed products.

Luyindula and Ledoux (1977), first reported results from experiments in which either soybean callus tissue or intact seedlings were subjected to DNA feeding experiments using radio-labelled materials. Those experiments indicated that exogenous DNA was rapidly degraded by callus tissue and the resulting degradation products were reutilized by the tissue. In germinating seedlings, soaked in bacterial DNA under aseptic conditions, nuclease activity was released by the roots, leading to the slow degradation of donor DNA in the medium, and the reutilization of degradation products for soybean DNA synthesis in a similar fashion to callus cultures. Autoradiographic studies demonstrated that twelve days after the experiment most of the exogenous DNA was associated with cell walls. In similar experiments involving protoplasts from synchronized soybean cell suspensions it was observed that radiolabelled bacterial DNA could be taken up by the protoplasts but this DNA was degraded rapidly due to high Dnase activities (Cress 1982).

Results from experiments describing the study of the interaction and binding between Agrobacterium tumefaciens and soybean cells in culture indicated that

bacteria were associated with the cell wall and often binding of bacteria was localized (Ohyama et al., 1979); however, soybean cells did not respond to infection with *Agrobacterium*, *in vitro* by the formation of tumors (Matthysse and Gurlitz, 1982).

The first successful experiments on the induction of tumors on soybean plants by *Agrobacterium* were reported by Pedersen et al. (1983); following induction of crown galls on soybean plants, the tumors were isolated and cultured *in vitro* as sterile callus and suspension cultures. Protoplasts isolated from suspension cultures could be regenerated to callus without any exogenously supplied hormones, thus confirming the tumorigenic nature of the tissues. Transformation was further confirmed by the production of opines and DNA blots. In an extension of this study the dynamics of endogenous IAA and cytokinins during the growth cycle of cloned crown gall cell lines were compared with wild-type soybean callus. Transformed tissues were found to contain 100-fold higher levels of zeatin glycosides than the wild type. No difference in the levels of auxin accumulation was detected between the two tissue types (Wyndaele et al., 1985).

Early attempts directed towards the development of an *Agrobacterium*-based vector for the introduction of exogenous DNA into soybean tissues quickly resulted in the realization that severe host-range restrictions existed which limited infectivity to very few specific genotypes. Lianzheng et al., (1984) studied the interaction of 15 *Agrobacterium* strains with 1,553 varieties of *Glycine max*, *G. soja* and *G. gracilis*. They reported that for soybean (*G. max*), out of 984 varieties screened, 25 developed tumors, some of which produced nopaline and exhibited hormone autotrophy. In a similar study, Owens and Cress (1985) screened 24 diverse cultivars of soybean and three lines of *G. soja* for their response to *Agrobacterium tumefaciens* and *A. rhizogenes*. Based upon gall weight at eight weeks post infection with *A. tumefaciens*, three cultivars were classified as highly susceptible, ten moderately susceptible, eleven weakly susceptible and two non-susceptible. Tumor lines were found to be hormone-autonomous, produced opines and DNA isolated from them hybridized with labeled T-DNA probes. Of 26 genotypes inoculated with *A. rhizogenes*, only seven responded in a clearly susceptible fashion by forming small, fleshy roots at internodal infection sites. Both groups demonstrated that soybean cells could be transformed with *Agrobacterium*, however, neither were able to regenerate any of the transgenic tumor lines to plants. Other investigators also reported results

from experiments involving *Agrobacterium* infection of seedlings (Facciotti et al., 1985; Byrne et al., 1987) and subsequent recovery of tumor lines producing opines, exhibiting hormone independent growth and kanamycin resistance. An alternative approach to increase the frequency of tumor formation and improve the host range of *Agrobacterium* involved the development of virulent bacterial strains (Hood et al., 1987). None of these approaches, however, resulted in the recovery of transgenic plants.

The situation with wild varieties of soybean appeared to be more promising. Rech et al. (1988, 1989) used *Agrobacterium rhizogenes* to induce transformed roots on *G. canescens* and *G. clandestina* which were subsequently regenerated into intact plants. The transgenic nature of these plants was confirmed by molecular and enzyme analyses. Unfortunately numerous attempts to utilize conditions proven effective for transformation of wild soybeans were unsuccessful with *G. max*, the cultivated soybean.

The first report describing the successful recovery of transformed soybean plants using *Agrobacterium* was reported by Hinchee et al. (1988). The procedure relies on regeneration in which shoot organogenesis is induced on cotyledons of a soybean genotype selected for susceptibility to *Agrobacterium* (cultivar Peking). Cotyledon explants from this specific cultivar were inoculated with *Agrobacterium*-harboring plasmids conferring kanamycin resistance and B-glucuronidase (GUS) activity, or kanamycin resistance and glyphosate tolerance. Following inoculation, explants were cultured on shoot-induction media containing kanamycin. Regenerated plantlets were tested for gene expression 3-4 months post-inoculation. Approximately 6% of the shoots produced on the kanamycin-selected cotyledons were transformed based on assays for GUS activity, kanamycin resistance or glyphosate tolerance. Progeny from two of those plants demonstrated cosegregation of the Kan^R phenotype with either GUS expression or glyphosate tolerance in a 3:1 ratio indicating a single insertion. Three parameters were critical in developing this particular soybean transformation protocol:

- (1) The use of the one cultivar (Peking) susceptible to *Agrobacterium* infection,
- (2) the development of a regeneration response from soybean cotyledons, and
- (3) the enrichment for transformed tissue by kanamycin selection.

A similar approach was used to demonstrate transposition of the maize controlling element (Ac) in transgenic soybean tissues of the cultivar Peking. Ac was inserted into the untranslated leader region of the bacterial GUS gene such that the excision of Ac resulted in restoration of the GUS gene activity.

Excision events of the *Ac* element were monitored by detecting blue cells or sectors in transgenic soybean tissues. Transposition was detected in transgenic soybean calli, leaves, stems and roots. However, no fertile transgenic plants or transformed progeny were obtained in these or subsequent experiments (Zhou and Atherly, 1990; Atherly, 1990).

Recovery of primary-chimeric transformants of soybean was accomplished using a somatic embryogenesis system in conjunction with *Agrobacterium* infection (Parrott et al., 1989). Again cultivar and bacterial strains were found to influence the recovery of transformed plants. Immature cotyledon tissues were co-cultivated with *Agrobacterium* carrying a 15kD zein gene. DNA from upper leaves of regenerated plants hybridized to a synthetic RNA probe specific for the input sequence. However, no transformed progeny was recovered from any of the putative primary transformants. The authors speculated that since the origin of the somatic embryos obtained in that system was multicellular, the resulting plants were chimeric and no germline transformation events had occurred. Additional reports involving either infection of germinating soybean seeds with *Agrobacterium* (Chee et al., 1989) or ovary microinjection methods (Liu et al., 1990) did not demonstrate convincingly the stable integration of exogenous DNA into the plant genome. In both cases dot blot hybridization and PCR analyses were the primary evidence for integrative transformation. These assays are known to be sensitive to background and false positive signals, so unless Southern blots are used for confirming integration of input DNA into high molecular weight fractions, these reports should be considered preliminary. In addition, mendelian segregation of progeny in the R2 generation should be demonstrated. These additional data are essential to confirm integrative transformation.

Soybean protoplasts can be transformed effectively using routine procedures. Various groups reported independently the recovery of stably transformed callus lines by introduction of plasmid DNA into protoplasts of soybean. Electroporation (Christou et al., 1987; Lin et al., 1987), or *Agrobacterium* co-cultivation (Baltes et al., 1987) were shown to be very effective in the recovery of transgenic soybean lines expressing a number of foreign genes such as *npt II*, *cat* or nopaline synthase. No plants have been recovered and regeneration still remains a formidable hurdle in the recovery of transgenic soybean plants from transformed protoplasts.

It is apparent from the above discussion that a common theme in soybean transformation by conventional approaches is the importance of finding varieties

that are both susceptible to *Agrobacterium* or protoplast culture, and capable of regeneration from transformed callus. Either one of these considerations severely restrict the range of varieties amenable to conventional transformation approaches. It is unfortunate, and for reasons not clearly understood, that varieties that fulfill one or more of these requirements do not include commercially important cultivars. A key requirement for the development of a useful and practical system for the modification of any crop, including soybean, is the ability to recover large numbers of independently derived transgenic plants in order to assess useful levels of gene expression. A sufficient number of independently-derived transformed plants is a prerequisite in order to compensate for varying patterns and level of expression of the introduced genes due to position effects. Time frames for the recovery of such plants should be rapid, in order to minimize or eliminate undesirable effects arising as a result of somaclonal variation in long term cell cultures. Finally, a genotype-independent transformation method is crucial for the effective introduction of agronomically useful traits into elite soybean varieties.

The development of the biolistics concept by Sanford (1988) and its reduction to practice in our laboratory, permitted the efficient transformation of soybean in a process that is truly cultivar-independent (McCabe et al., 1988. Christou et al., 1990). The process is based on the introduction of heavy metal particles coated with plasmid DNA into intact and regenerable plant tissues. Prior to our work with soybean, Klein et al. (1987) demonstrated that high velocity tungsten particles coated with DNA could be introduced into intact epidermal onion cells. The cells expressed transiently the introduced DNA. Questions remained, however, as to whether this novel technique would result in the stable integration of plasmid DNA into plant tissues. The first question we were attempting to answer was the following: "Was the biolistics process capable of delivering biologically active DNA into plant cells and if so, would this DNA be stably integrated into the plant's genome?" Using a previously developed protoplast regeneration system (Christou et al., 1988) we were able to recover callus lines expressing foreign genes by bombarding immature embryo cotyledons and isolating protoplasts from them. These protoplasts could be cultured under selection conditions and regenerated to give stably transformed callus lines expressing the introduced genes. Roots regenerated from the callus lines also expressed the foreign genes. We considered these results to be very important for a number of reasons: (1) We had demonstrated that the biolistics process or

variations thereof, could result in stable integrative transformation of plant tissues, (2) transformation frequencies were of the same order of magnitude as electroporation or other direct DNA transfer methods, (3) intact soybean tissue, rather than callus could express heterologous genes. The next step in the process was to bombard regenerable tissue. We had previously reported a prolific system for the regeneration of soybean using somatic embryogenesis from immature embryo cotyledons (Christou and Yang, 1989). These explants were bombarded under a wide range of conditions and somatic embryos were recovered. However, despite many attempts not a single transformed plant was recovered from this procedure. Alternative explants were subsequently investigated as candidates for transformation. We determined that embryonic axes isolated from immature seeds of greenhouse grown soybean plants were the best candidates for particle acceleration experiments. In subsequent experiments we found that embryo axes from dry seeds could also be used but transformation frequencies were reduced. Thus, by combining a simple genotype-independent regeneration protocol based on the proliferation of multiple shoots from the general area of the meristem of soybean embryonic axes, and electric discharge particle acceleration for the delivery of foreign DNA we were able to develop a commercial process for the introduction of any gene into any soybean variety (Christou et al., 1990). Many hundreds of independently derived soybean plants have been recovered and analyzed to date. Most plants were found to be chimeric. This is not too surprising considering the nature of the DNA delivery method. Degrees of chimerism were studied following expression of the *gus* gene in regenerating plantlets. By studying the morphology and phenotype of such chimeric plants we were able to reconstruct a partial picture for the development of the soybean plant (Christou, 1990). A number of plants recovered in these experiments appeared to express the introduced genes in all their cells. Extensive molecular and genetic analyses demonstrated that these plants were derived from a single cell and were clonal in nature (Christou et al. 1989) segregating in a mendelian fashion in the R1 and subsequent generations. A number of empirical rules were developed based on our experience with transgenic soybeans: (1) Each transformant is unique; (2) Transformed plants usually contain one to several copies of the introduced gene. Plants containing very large numbers of the foreign genes are obtained, but are relatively rare (10% or less); (3) All copies of input genes are usually genetically linked; (4) Each transformant likely derived from a single transformation event; (5) Input genes physically linked on the same plasmid are

recovered together in >90% of the transformed plants. Genes introduced on separate plasmids are recovered together in only 20-25% of transformed plants (Christou and Swain, 1990); (6) Genes are stably inherited and usually show normal mendelian inheritance. Aberrant segregation due, in some cases, to pollen inviability is obtained at frequencies higher than expected; (7) Chimeric R0 plants can give rise to R1 progeny. R2 and subsequent generations segregate in mendelian manner; (8) Homozygous lines can be demonstrated in the R2 generation.

The above empirical observations permitted the development of an efficient and unique process for the commercialization of elite varieties of soybeans expressing agronomic traits. Soybean plants generated in our laboratory that express tolerance or resistance to the herbicides glyphosate and basta, respectively, are currently being evaluated in field trials.

The development of an efficient transformation system for soybean allows a shift of research effort away from developing tissue culture systems, and labor-intensive and time-consuming transformation procedures for the introduction of foreign genes into this crop. The emphasis of basic research in the area of soybean improvement should now be directed towards the isolation and characterization of genes that control agronomically useful qualitative and quantitative traits. Introduction of herbicide resistance genes has been accomplished and practical levels of herbicide tolerance are likely to be demonstrated soon. Marketing of genetically engineered soybeans expressing herbicide resistance will be realized in the next few years. Efforts to modify seed storage proteins for improved nutritional value for human food and animal feeds are currently underway in our laboratory. Targets such as disease and drought resistance, as well as tolerance to other stress factors that influence improved productivity will be the next challenge, the ultimate goal, of course, being yield increase. Modification of oil composition to suit diverse and specific applications will become a realistic target for soybean improvement as key enzymes involved in fatty acid and triglyceride biosynthesis are identified and the corresponding genes isolated and characterized. Exciting progress in these areas has been achieved over the last several years and should proceed at accelerating rates provided that the research effort is adequately supported.

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Paul Christou

UNITED STATES DEPARTMENT OF AGRICULTURE
 Agricultural Research Service
 Plant Molecular Biology Laboratory
 Plant Sciences Institute
 Beltsville MD 20705

1) Tests for genetic linkage or independence.

Tests for genetic linkage were conducted both in the field and in the greenhouse. The gene symbols given in Table 1 refer to the genetic traits described by Palmer and Kilen (1987). Recombination was calculated from F2 or F3 data using the method of maximum likelihood as described by Allard (1956) and Mather (1951). The bisection method (Yakowitz and Sidarowszky, 1989) was used to solve the maximum likelihood equations. As previously reported (Devine et al., 1983), the rj1 and f loci were linked. None of the other combinations tested showed definitive evidence of linkage. The pc locus and y9 locus had a recombination value of $41 \pm 4\%$; however, in an earlier study (Devine and O'Neill, 1989) the recombination value for these loci was $54 \pm 3.2\%$. The Pl and Yl0 loci had a linkage chi-square value significant at the 5% level; however, the recombination value was $45 \pm 2.2\%$ indicating, at most, only a very distant linkage.

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Table 1. Soybean genetic linkage tests.

cross #	genes		genotype																	Sum	X2L	Prob	R	SE	phase	ratio
	A	B	C	D	E	F	G	H+I	J	K	L	M	N													
Barc-1 X T210	<i>d2,f</i>	112 43	47	13																215	0.87	.3-.5	45	5	R	9331
	<i>d2,</i>	124 31	43	17																215	1.56	.2-.3	1+	5	R	9331
	<i>r11</i>																									
Barc-1 X T141	<i>pc,r11</i>	344 95	80	31																550	1.94	.2-.1	45	3.0	C	9331
	<i>f,pc</i>	324 100	87	39																550	2.63	.05-	44	2.9	C	9331
																					.1					
Barc-1 X T230	<i>f,r11</i>	352 87	59	52																550	29.5	.001	33	2.5	C	9331
	<i>f,r11</i>	304 69	94	48																515	13.2	.001	38	2.8	C	9331
	<i>f,y13</i>	300 98	88	29																515	.002	.9-1.	50	3.3	R	9331
	<i>f,i</i>	150 69	43	25																287	.589	.3-.5	53	4	R	9331
	<i>r11,i</i>	134 74	59	20																287	2.80	.05-	43	5	R	9331
																					.1					
Barc-4(Rj2) X BV-4	<i>r11,y</i>	283 90	105	37																515	.207	.5-.7	51	3.3	R	9331
	<i>i,y13</i>	146 68	47	26																287	.474	.3-.5	47	4	C	9331
	<i>rj2,</i>	192 53	72	25																342	.573	.3-.5	46	4	C	9331
BV-1 X Barc-4(Rj2)	<i>y9</i>																									
	<i>pl,</i>	750 262	260	64																1336	4.79	.01-	45	2.2	R	9331
	<i>y10</i>																				.05					
BV-4 X T141	<i>f,pc</i>	201 64	62	13																340	1.18	.2-.3	56	4	R	9331
	<i>f,y9</i>	203 60	63	14																340	.52	.3-.5	54	4	C	9331
	<i>pc,y9</i>	214 51	52	23																340	4.10	.01-	41	4	C	9331
																					.05					
Kura X Barc-1	<i>f,r</i>	119 54	63	25																261	.02	.7-.9	50	4	C	9331
	<i>r,r11</i>	124 49	68	20																261	.940	.3-.5	54	4	C	9331
	<i>f,r11</i>	143 39	49	30																261	9.45	.001	39	5	C	9331
Kura X Barc-1																					.01					
	<i>f,i-k</i>	141 57	41	22																261	.862	.3-.5	54	4	R	9331
	<i>r,i-k</i>	136 37	62	26																261	2.27	.1-.2	56	4	R	9331
	<i>i-k,</i>	142 56	50	13																261	1.48	.2-.3	44	5	R	9331
	<i>r11</i>																									

Lines prefixed by T are from the soybean genetic type collection (Palmer and Kilen, 1987) maintained by R.L. Nelson, ARS, USDA, Agronomy Department, University of Illinois. P1290136 is a plant introduction accession obtained from the same source. BV-1 is a line, constructed at Beltsville from T161 and T145, carrying *P1* and *y10*. BV-4 is a line also constructed at Beltsville from the cross of T135 X P183945-4 and carries the *f* and *y9* alleles. BARC-1, carrying *f* and *r11*, and BARC-4 (*Rj2*), carrying *Rj2*, were constructed at Beltsville and described in Crop Science 26:1091, and Crop Science 27:1322-1323, respectively.

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T. E. Devine

THE UNIVERSITY OF TENNESSEE
 Plant Molecular Genetics and
 Center for Legume Research
 Knoxville TN 37901-1071
 and
 USDA/ARS
 Iowa State University
 Ames IA 50011

1) The *nts* locus controlling supernodulation in soybean is next to RFLP marker pA-132.

Some years ago we discovered that soybean can supernodulate (Carroll et al., 1985a,b). The isolation of *nts* (supernodulating) mutants in cultivar 'Bragg' started a renewed interest in the analysis of the plant's contribution to the nitrogen-fixing root nodule symbiosis.

Since then we have accumulated a large database describing the *nts* material. Much of this has been review (see Caetano-Anolles and Gresshoff, 1991; Carroll and Mathews, 1990; Rolfe and Gresshoff, 1988; Gresshoff and Delves, 1986).

The essential facts about the *nts* mutations are as follows. A total of 15 *nts* isolates were selected from M2 families after EMS mutagenesis (Carroll et al., 1985a,b; 1986). Of these, 12 survived and were described in more detail. Of these isolate *nts*382, *nts*116, and *nts*1007 were most thoroughly researched. All mutant alleles were found to be single mendelian recessives (Delves et al., 1988; Carroll et al., 1988). All alleles are in the same genetic complementation group, despite apparent phenotypic differences (Delves et al., 1988). Supernodulation was epistatically suppressed in double mutants carrying the *nts* and *nod* minus alleles (Mathews et al., 1990). The *nts* locus was found to be unlinked from the *nod* alleles and the locus controlling purple flower color (Mathews et al., 1990). The supernodulation phenotypes result in nitrate tolerant nodulation and an apparent absence of the systemic inhibition process controlling nodulation (Olsson et al., 1989).

Surprisingly, we discovered that the supernodulation phenotype is controlled by the shoot (Delves et al., 1986, 1987a,b). More recently we recognized that the autoregulation suppression, diminished in the *nts* mutants,

requires only the leaf and not the shoot apex (Delves et al., 1991). Histological analysis showed that supernodulation plants have diminished ability to suppress cell division clusters in the cortex during pre-nodule development (Mathews et al., 1989a). The analysis of root exudates from young seedlings demonstrated that the nod gene inducing ability of mutant nts382 was equivalent to that of wild-type (Sutherland et al., 1990; Mathews et al., 1989b). Isotope dilution studies by Hansen et al. (1989) indicated that nts1007 has a significant high ability to fix atmospheric nitrogen in the presence of externally supplied nitrate. Likewise nts382 was found to be more tolerant of the inhibitory effects of soil acidity (Alva et al., 1988). More recently we discovered that the autoregulation mechanism controlling nodulation in soybean is apparently activated by the cell division pre-nodule or events closely related to it (Caetano-Anolles and Gresshoff, 1990).

Since nothing is known about the gene products controlled by the nts locus (see Sayavedra-Soto, et al., this volume), one finds that conventional methods used to isolate a gene are not applicable to the nts system. For this reason we chose to attempt a gene isolation which is based at first at the mapping of the gene in question. This was facilitated through the recent development of an RFLP map for soybean (Keim et al., 1990). Future steps will involve the isolation and cloning of large molecular weight fragments of soybean genomic DNA, their eventual comparison as template DNA for short primer-driven DNA amplification fingerprinting (DAF; see Caetano-Anolles et al., 1991; and this volume), and potential genetic transformation of homozygous recessive mutant plants with dominant wild-type sequences. We here report the accomplishing of the first step., i.e., the close RFLP mapping of the nts locus. A more detailed description is given by Landau-Ellis et al. (1991).

This description is of relevance also to other soybean geneticists as recently and independently several additional nts mutants of soybean were isolated (Gremaud and Harper, 1989; Buzzell et al., 1990; Akao and Kouchi, 1991).

Materials and Methods: Glycine max (L.) Merrill line nts382 (Carroll et al., 1985a) was crossed to Glycine soja PI 468,397 (kindly supplied by Dr. Gary Stacey, CLR, University of Tennessee, Knoxville) under field conditions. F1 and F2 progeny were grown in the greenhouse inoculated with Bradyrhizobium japonicum strain USDA110, grown in liquid medium (using about 10^{10} bacteria per

plant).

RFLP mapping: The USDA-ARS/ISU map (Keim et al., 1990) was produced by analyzing on average 60 plants of an F2 population from a cross between Glycine max (line A81-356,022) and G. soja (PI 468,961). Polymorphic clones are part of a PstI library in the pBS+ plasmid from Stratagene (Keim et al., 1990). About 30% of all clones detected polymorphisms in the parental material. Not all RFLP probes derived from this bank showed RFLPs with the genetic lines used for this study. Therefore, all mapped markers were first confirmed for their ability to detect clear RFLPs in nts382 and PI 468,397. Line nts382 (in a soybean cultivar 'Bragg' background) was crossed with G. soja accession PI 468,397, using the symbiotic mutant as the female. F1 plants were confirmed after 4-5 weeks for their nodulation phenotype. DNA analysis verified assignments as hybrid RFLP patterns were determined. Verified F1 plants were allowed to self-fertilize and F2 plants were grown in synthetic growth medium inoculated with Bradyrhizobium japonicum strain USDA110, and scored after 4-5 weeks for their nodulation phenotype. Our isolation procedure selected directly the supernodulating segregants from the F2 population. Our strategy then demanded the detection of cosegregation with a set of randomly selected RFLP markers. F2 plants, being homozygous recessive for nts, were used to isolate total genomic DNA, using methods described elsewhere (Dellaporta et al., 1983). Purified DNA was restricted with appropriate endonucleases, separated by agarose gel electrophoresis (20 ug/lane, 0.9% agarose), and transferred onto Zeta Probe Nylon membrane (Bio-Rad). Membranes were probed with radioactively (adCT³²P, random primed method) labelled inserts from pBS+ clones known to be polymorphic between the two parents. Hybridizations were performed at 60°C. Membranes were exposed to X-ray film for varying amounts of time.

Results and Discussion: The soybean RFLP linkage map (USDA-ARS) now contains about 300 polymorphic markers, spanning about 2800 centimorgans (cM) at an average distance of 9.3 cM (Keim et al., 1990).

Sexual hybridization of line nts382 (supernodulating, erect habit, white flower and green hypocotyl, yellow seed, large seed) with G. soja accession PI 468,397 (wild-type nodulation, viney habit, purple flower and hypocotyl, black seed, small seed) gave frequencies between 1 to 10%, and reflected experimental and environmental conditions at the time of crossing. F1 hybrids were confirmed by their nodulation phenotype (wild type, because nts382 was

the female parent), RFLP banding patterns (Fig. 1), flower color (purple is dominant to white), hypocotyl color (purple is dominant over green), growth habit (intermediate vining), and seed size (intermediate to small) and color (intermediate, fawn). From a total of 82 F₂ plants, 20 possessed the nts phenotype, confirming the expected Mendelian segregation ratio (3 wild type : 1 supernodulator). These supernodulating plants were repotted and used for plant DNA isolation. Genomic DNA isolated from young leaves of individual F₂ plants was analyzed for the co-segregation of an RFLP marker and the supernodulation phenotype.

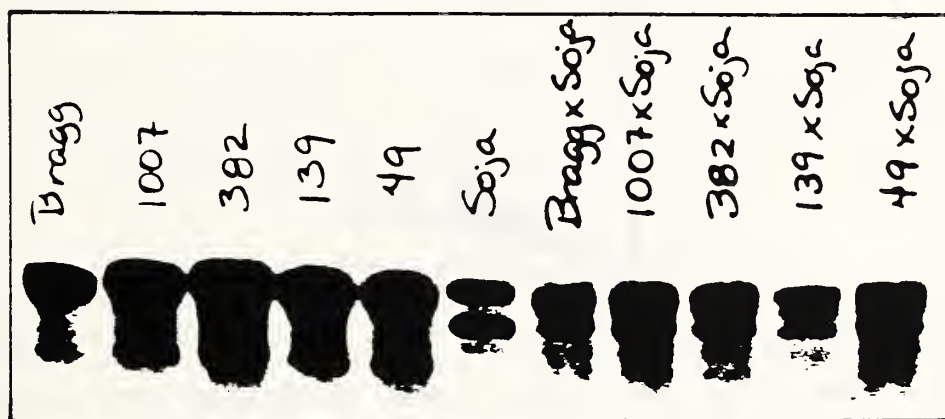


Figure 1. RFLP pattern detected by probe pK-19 in hindIII digested genomic DNA from Glycine max cv. Bragg, its supernodulation and non-nodulation mutants and the F₁ hybrids with Glycine soja.

Probe pA-36 from tentative linkage group E showed linkage with 10% recombination, while clones from other linkage groups such as probe pK-19 assorted independently. Linkage group 'E' is characterized by well-spaced marker pA-703 (giving a distance of approximately 37 cM, Table 1) and pA-132. Probe pA-132 showed no recombination with the nts locus, giving only the G. max RFLP pattern. However, the insert in pA-132 was found to contain three separate pstI inserts, thus resulting in a complex banding pattern (not shown here). We determined that the largest insert (1.75 kb) was the fragment detecting the linked polymorphism. When this insert was used as a probe, a simple pattern was detected showing no recombination and therefore 100% linkage in the nts F₂ segregants. This insert band was recloned and is now

identified as pUTG-132a.

The presence of the weakly hybridizing band below the polymorphic band in the nts382 digest at first may confuse the distinction between the G. max and the possible hybrid. However, both band intensity and band position can be distinguished, allowing us to conclude that only nts382 types were analyzed.

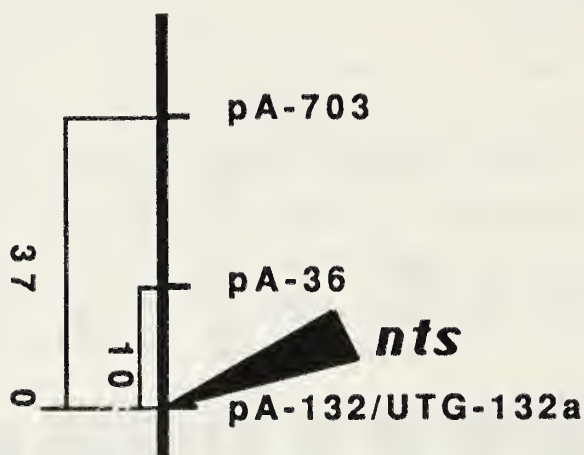


Figure 2. Part of tentative linkage group E from the USDA/ARS RFLP map showing the location of the nts locus.

Calculation of genetic distances demonstrates that they are nearly additive (Table 1), and that the nts locus is located in a region of the soybean genome that may not have major distortions of recombination. Markers pA-89a, pK-9d and pA-69 were not used to map in this study, as pA-36 was representative for that region.

A parallel map has been produced by the DuPont Company, and no attempt was made to place nts on that map.

Table 1. Linkage verification of *nts* locus in soybean.

Marker pair	RFLP pattern (max:hybrid:soja)	Percent recombination	χ^2 (0.05)
<i>nts</i> 382/pA-36 ¹	15 : 4 : 0	10	0.30
<i>nts</i> 382/pA-703 ²	6 : 8 : 3	37	0.04 ³
<i>nts</i> 382/pUTG-132a	20 : 0 : 0	0	0.00
pA-36.pA-132	from map	18.8(13.2)	----
pA-36/pA-703	from map	15.0(17.8)	----

¹Only 19 of the 20 *nts* segregants were scored. ²Only 17 plants of the 20 *nts* segregants were scored. Recombination values in brackets signify a possible reversal of gene order, which is unresolvable with present sample numbers and low recombination distances in the pA-36 cluster. ³This value gives the best fit, but the hypothesis for independent segregation could also be accepted.

The linkage to pA-36 and the fact that only a small number of F2 plants was scored for the construction of the map, suggests the possibility that the pA-36 cluster may in fact be in an inverted orientation on the actual soybean chromosome. We postulate the linkage arrangement to be as shown in Figure 2.

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Peter M. Gresshoff
 Deborah Landau-Ellis
 Sieglinde Angermuller
 University of Tennessee

Randy Shoemaker
 Iowa State University

UNIVERSITY OF MISSOURI DELTA CENTER

Portageville MO 63873

1) Additional dominant gene in PI 88,788 conferring resistance to soybean cyst nematode race 3.

The inheritance of resistance in soybean to soybean cyst nematode (SCN) damage caused by Heterodera glycines Ichinohe is complex. Early reports based on SCN field populations of North Carolina and Missouri indicated that resistance in 'Peking' was conditioned by three independent recessive genes (rhg₁, rhg₂, rhg₃), and one dominant gene (Rhg₄), respectively (Caldwell et al. 1960; Matson and Williams, 1965). These SCN populations were later categorized to be Race 1 and Race 3, respectively. In Peking the dominant gene found was linked with its black seed coat color (Matson and Williams, 1965).

Additional genes in Peking conditioning resistance to SCN Race 3 have not been described. A few genetic studies reported on Peking concluded that the genes were in common for SCN Race 3 resistance in Peking, cv. 'Forrest' and PI 90,763 and the crosses between Peking x PI 88,788 have segregated in F₂ and F₃ generations (13R:3S), indicating dominant recessive epistasis (Rao-Arelli and Anand, 1988a, 1988b). The objective was to determine inheritance of resistance in Peking for reaction to an isolate of SCN Race 3 and examine the relationship of Rhg₄, and any other genes occurring in it to those genes already reported in PI 88,788 (Rao-Arelli et al., 1988).

Materials and Methods: Seeds of soybean lines used in this report were obtained from R. L. Bernard, USDA-ARS, University of Illinois, Urbana-Champaign. The following crosses were made at the University of Missouri-Delta Center, Portageville, during 1986 and 1987 summers.

- (1) Peking (Resistant - R) x cv. Essex (Susceptible - S)
- (2) PI 90,763 (R) x cv. Essex (S)
- (3) PI 90,763 (R) x PI 88,788 (R)

F₁, F₂ and F₃ plants of each cross were evaluated against Race 3 as described by Rao-Arelli and Anand (1988a). The rapid inoculation techniques used in this report were also published (Rao-Arelli et al., 1990). The index of parasitism (IP) for each plant was calculated. An IP of $\geq 10\%$ was classified as susceptible, whereas an IP of $< 10\%$ was classified as resistant (Golden et al., 1970). Chi-square analysis was used to test goodness of fit based on the

segregation of one or more genes.

Results and Discussion: The F_1 hybrids of the cross Peking (R) x cv. Essex (S) were susceptible (Table 1). The F_2 population segregated into 16 resistant and 293 susceptible. Based on a two-gene model, the segregation fit a 1:15 ratio ($p = 0.3 - 0.5$), and a three-gene model of 3:61 ($p = 0.5 - 0.7$), respectively (Table 1). In the F_3 generation, one family was resistant and 67 were either susceptible or segregating. This was a good fit to the expected dihybrid ratio of 1:15 ($p = 0.1 - 0.2$) and a trihybrid ratio ($p = 0.7 - 0.9$), respectively. Based on the P value, the data fit very closely to one dominant and two recessive genes for SCN Race 3 control in Peking.

The F_1 plants from the cross PI 90,763 (R) x Essex (S) were susceptible to SCN Race 3 (Table 1). The cross segregated into 14 resistant and 180 susceptible plants in the F_2 generation. Two different gene models were proposed to explain segregation. A two-gene model included two recessive genes (1R:15S; $P = 0.5 - 0.7$) and a three-gene model included one dominant and two recessive genes (3R:61S; $P = 0.05 - 0.1$), respectively, for conditioning resistance in PI 90,763. In the F_3 generation, two families were resistant and 69 were either susceptible or segregating for the cross PI 90,763 (R) x Essex (S). This was a good fit to the expected dihybrid ratio of 1:15 ($P = 0.1 - 0.3$) or a trihybrid ratio of 1:63 ($P = 0.3 - 0.5$), respectively.

The cross PI 90,763 (R) x PI 88,788 (R) segregated into 227 resistant and 42 susceptible in the F_2 generation (Table 1) for SCN Race 3. The segregation ratio was a good fit to 13R:3S ($P = 0.1 - 0.2$) with a dominant and recessive epistasis. In the F_3 generation, 30 families were either resistant or segregating against three susceptibles. This was consistent with the expected ratio of 15R:1S ($P = 0.5 - 0.7$).

A three-gene model in each of three parents alone can explain the resulting ratio of 13R:3S in Peking x PI 88,788, and PI 90,763 x PI 88,788 segregations due to dominant and recessive epistasis. Genes S, s were assumed for susceptibility and Rhg, rhg for resistance to SCN Race 3, respectively. The following were the proposed general genotypes for the parents:

PI 88,788 (R) :	<u>S</u>	<u>S</u> ,	<u>rhg</u>	<u>rhg</u> ,	<u>Rhg</u>	<u>Rhg</u> ,	<u>Rhg</u>	<u>Rhg</u>
Peking (R) :	<u>rhg</u>	<u>rhg</u> ,	<u>rhg</u>	<u>rhg</u> ,	<u>s</u>	<u>s</u> ,	<u>Rhg</u>	<u>Rhg</u>
PI 90,763 (R) :	<u>rhg</u>	<u>rhg</u> ,	<u>rhg</u>	<u>rhg</u> ,	<u>s</u>	<u>s</u> ,	<u>Rhg</u>	<u>Rhg</u>
Essex (S) :	<u>S</u>	<u>S</u> ,	<u>S</u>	<u>S</u> ,	<u>s</u>	<u>s</u> ,	<u>s</u>	<u>s</u>

A single dominant (Rhg) and a single recessive (rhg) resistance gene are in common between parents, PI 88,788, Peking and PI 90,763. Presumably, this dominant gene in Peking was the one previously designated (Rhg).

A second recessive gene (rhg) in both parents, Peking and PI 90,763, and an additional dominant gene (Rhg) in PI 88,788 appear to have provided an epistatic interaction. Segregating populations with rhg rhg S - (3/16) genotypes were thus susceptible in crosses of either Peking x PI 88,788 or PI 90,763 x PI 88,788. An additional dominant gene for SCN Race 3 resistance is occurring in PI 88,788.

Two of the three recessive resistance genes designated in Peking to SCN Race 1 also appeared to condition resistance to SCN Race 3. Perhaps the third recessive gene in Peking might be conferring resistance to other races of SCN.

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Table 1. Reaction of F_1 , F_2 plants, and F_3 families from crosses between different soybean genotypes to SCN Race 3 isolate.

Number	Cross	R/S	Observed		Expected		X ²	P	Genetic ratio
			R	S	R	S			
Peking x Essex									
1.	(F ₁)	10(S)							
2.	(F ₂)	16	293		14.5	294.5	0.16	0.5-0.7	3(R):61(S)
3.	(F ₃)	1	67#		1.1	66.9	0.01	0.7-0.9	1(R):63(S)
PI 90,763 x Essex									
4.	(F ₁)	10(S)							
5.	(F ₂)	14	180		9.1	184.9	2.78	0.05-1	3(R):61(S)
6.	(F ₃)	2	69#		1.1	69.9	0.75	0.3-0.5	1(R):63(S)
PI 90,763 x PI 88,788									
7.	(F ₂)	227	42		218.5	50.5	1.76	0.1-0.2	13(R):3(S)
8.	(F ₃)	30#	3		30.9	2.1	0.40	0.5-0.7	15(R):1(S)

- Include segregating families.

R - Resistant; S - Susceptible.

A.P. Rao-Arelli

S.C. Anand

J.A. Wrather

UNIVERSITY OF ILLINOIS

AT URBANA-CHAMPAIGN

Department of Agronomy

1102 South Goodwin

Urbana IL USA 61801

1) A new chlorophyll mutant in soybeans.

Introduction: There are presently 36 chlorophyll mutants in the soybean genetic type collection. These mutants vary in leaf morphology (i.e., color, variegation), are often lethal, and may be influenced by environmental factors such as temperature fluctuations. We report on a new chlorophyll mutant that is lethal under field conditions.

Materials and Methods: In the late spring of 1990 several chlorophyll-deficient plants were observed in an F3 breeding population (designated LNX8716). Affected plants were light yellow and died approximately 15 days after emergence. To further study this mutant, F3 seed was planted in the greenhouse. Plants expressing the mutation were pale yellow and developed necrotic brown spots after the V2 growth stage. Although these plants did not die they were unable to set pods. To determine if the mutation was novel, a visual comparison was conducted in the greenhouse between the LNX8716 mutant and chlorophyll mutants in the germplasm collection.

To evaluate the inheritance of the chlorophyll mutation, 192 plants from the LNX8716 population were harvested and threshed separately. Ten F3:4 seeds from each of the 192 F3 plants were planted in the greenhouse and scored for expression of the chlorophyll mutation. After recording the data a chi-square test was conducted to determine the goodness of fit to a single gene model.

Results and Discussion: Of the 192 families, 70 segregated for the chlorophyll-deficient mutant while the other 122 F3 families showed no segregation (Table 1). These results appear to support the hypothesis of a recessive monogenic type inheritance (Table 1). Comparison of the LNX8716 mutant with chlorophyll mutants in the germplasm collection revealed few

morphological similarities. Lines T116H and T225H revealed the greatest resemblance and will be utilized to conduct an allelism study in the summer of 1991.

Table 1. Inheritance of LNX8716 mutant.

Generation	Observed ratio	Expected ratio	Chi square	P
F3 families	122:70	114:78	1.38	0.50-0.90

M. J. Graham

C. D. Nickell

2) A pink flower-color mutant in soybean.

Introduction: Most soybean cultivars have either purple or white flowers (Woodworth, 1923). Dilute-purple and near-white flowers have been described (Hartwig and Hinson, 1962) but these flowers appear white and identification requires close examination of the separated standard. A magenta flower color mutant has been reported by Buzzell et al. (1977). We report a new flower color, described as pink, which is quite distinct from all previously reported flower colors. Pink flowers are uniform in color throughout the petals, in contrast to purple, magenta, dilute-purple, and near-white flowers which have increased pigmentation in the veins when compared to the petals.

Materials and Methods: This pink flower was first observed during the summer of 1989 in two F4:5-derived plant rows (designated LN89-5320 and LN89-5322) segregating for purple and pink flower pigmentation while homozygous for gray pubescence and imperfect black hilum. These lines had originated from the cross ('Sherman' x 'Asgrow A2943') x 'Elgin 87'. All F4 plant selections from

this cross were purple in flower color. For the two F4:5 plant rows having the pink flower mutation, F5 plants in both rows were classified as pink or purple in flower color, harvested individually, and planted in the spring of 1990.

To determine if the pink flower trait is different in appearance from known flower color genes, pink flowered plants were planted alongside 'Clark' and 'Harosoy' flower color isolines (Bernard, 1978; Table 2).

Results and Discussion: Segregation data for flower color in 1989 suggested that the pink flower mutation was a single gene recessive trait (Table 1). Segregation for flower color during the summer of 1990 confirmed that the pink flower color was controlled by a single gene (Table 1). The 16 F4:5 purple plants segregated in a 1:2 ratio (homozygous:heterozygous) as expected and the 10 heterozygous rows segregated 3:1 (purple-pink) as expected

Table 1. Combined flower color data for LN89-5320 and LN89-5322.

	Flower color			Ratio	Chi square Probability
	Purple	Seg. No.	Pink		
1989 plants	16(9+7)#		7(4+3)	3:1^	0.55 (0.63, 0.72)
1990 F4:6 plant rows	6	10	7	1:2:1@	0.78
1990 segregation for F4:5 purple plant	6	10		1:2	0.72
1990 individual plant data for 10 segregating rows	350		128	3:1	0.37

#Data in parentheses for LN89-5322 and LN89-5320, respectively

^Ratio = purple to pink

@Ratio = purple to segregating pink.

Table 2. Clark and Harosoy isolines for flower color.

Designation	Genotype#	Phenotype
Clark 63	<u>W1 w3 W4 Wm</u>	purple
L70-4422	<u>W1 W3 w4 Wm</u>	purple throat [^]
L68-1774	<u>W1 w3 w4 Wm</u>	near-white
L72-2181	<u>W1 w3 W4 wm</u>	magenta
L69-4776	<u>w1 w3 W4 Wm</u>	white
Harosoy 63	<u>W1 w3 W4 Wm</u>	purple
L72-1078	<u>W1 W3 w4 Wm</u>	purple throat
L72-1138	<u>W1 w3 w4 Wm</u>	near-white
T235	<u>W1 w3 W4 wm</u>	magenta
L64-2139	<u>W1 w3 W4 Wm</u>	white

#Lines are homozygous for flower color genotype.

[^]Purple throat = dilute-purple.

for single gene inheritance. It is possible that LN89-5320 and LN89-5322 were from the same F4 plant harvested in 1988 (a branch having broken away and threshed as a second plant).

During the growing season it was readily apparent that none of the Clark and Harosoy flower color isolines resembled the pink flowered mutant. Crosses have been made between the pink flowered mutant and the Clark isolines. Future work will determine the allelic and phenotypic interactions with the known flower color genes.

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P. A. Stephens

C. D. Nickell

3) Chemical mutagenesis of T31 soybean.

Introduction: T31 is an unusual variant of Glycine max (L.) Merrill. It was discovered by Stewart and Wentz (1926) who reported several distinctive characteristics of the plant. T31 has no obvious pubescence (puberulence). The puberulent trait is inherited recessively in a simple Mendelian manner (Stewart and Wentz, 1926; Woodworth and Veatch, 1929; Bernard and Singh, 1969), and has the genetic symbol p2 (Stewart and Wentz, 1926).

T31 also has a defective seed coat that is brown. The entire surface of the seed is covered by a network of cracks. The degree of defectiveness varies among seeds within plants and among plants, and depends on environmental conditions (Bernard and Singh, 1969). This characteristic makes T31 agronomically undesirable.

Several attempts have been made to measure the linkage between defective seed coat and puberulence. The first study, done by Woodworth and Williams (1938) reported 2% crossover. They assigned the genetic symbol de to the defective seed coat trait. However, later studies revealed no crossover between the two traits, leading to the conclusion that p2 and de are pleiotropic effects of the same gene (Bernard and Singh, 1969; Weiss, 1970). Williams subsequently acknowledged that the crossover data was based on only four crossover plants and was not substantiated by further progeny tests (Bernard and Singh, 1969). Both traits have been assigned to Linkage Group IV (Weiss, 1970).

The most unusual trait of T31 is its exceptionally high degree of natural outcrossing. Rates of 10% have been reported in the US (Bernard and Jaycox, 1969), and rates of up to 40% have been reported in India (Singh, 1972).

These three traits of T31 raise several interesting theoretical and practical issues. Puberulence could be a useful marker gene in crossing attempts. If T31 is used as the female parent, successful crosses can be identified in the early seedling stage because p2 is a recessive trait. Practically, however, it would be more useful if puberulence could be separated from defective seed coat.

The high rate of natural outcrossing could be used to replace the tedious procedure of making hand cross-pollinations. In combination with the

recessive puberulence trait it would provide a simple system for making and identifying a cross.

In previous work to determine the genetic relationship between puberulence and defective seed coat, traditional crossing methods were used. In the current study chemical mutagenesis was used in an attempt to break the linkage between the two traits.

Materials and Methods: Some 3,700 field-grown T31 seeds were treated with nitrosomethyl urea (NMU) a chemical mutagen. The method used was described by Ryan and Harper (1983) and involved a 3-h treatment with 2.5 mM NMU followed by a 10-h postwash period. Immediately after the mutagenesis/postwash treatment the seeds were planted in a sand bench. All seeds that germinated (636) were transplanted to individual 6-in clay pots and grown to maturity in the greenhouse. Seeds harvested from the M1 plants were planted in individual 6-in pots and grown to maturity in the greenhouse. If a M1 plant produced less than 15 seeds, all its seeds were planted. If a plant had 15 seeds or more, at least 15 seeds were planted. Observations were recorded for all M2 plants. Approximately 16,000 seeds were harvested from the M2 generation. Seeds from 25 M2 plants lacked defective seed coat and these seeds (248) were grown in the greenhouse. Observations were made on the seeds produced by each plant. Of the 248, 18 plants produced seed without defective seed coats. Seeds from these 18 M3 plants were advanced to the M4 generation. Of the approximately 500 seeds planted from M3 plants, 32 plants produced seeds without defective seed coats. The pedigrees of these M4 plants are shown in Table 1.

Results and Discussion: Of the 3717 T31 seeds that underwent the NMU treatment, 636 germinated into seedlings. The 17% germination was lower than expected on the basis of 46% germination of similarly mutagenized Williams seed (Ryan and Harper, 1983). It may be that the defective seed coat allowed greater penetration of the chemical mutagen and hence was more lethal for the T31 seed.

Normal pubescence was seen on 41 of the M1 plants. However, all of the pubescent plants were subsequently found to be yellow seeded and thus were eliminated and assumed to be natural crosses from the previous generation, which was grown in the field. No normal seed coats were found on seed from the puberulent plants of the M1 generation.

All of the M2 plants derived from seed of puberulent M1 plants were puberulent. Twenty-five M2 generation plants produced seeds that did not display the defective seed coat, i.e., they were smooth and brown. Seeds from these 25 M2 plants were grown in the greenhouse. Eighteen of these M3 plants from seven original M1 parents produced apparently normal brown seeds. Table 2 summarizes the ratios of smooth-seeded plants to total number of plants for the seven M1 families that continued to segregate. In the M4 generation 32 out of 500 plants did not express the de trait.

If any of these M4 plants represents a break in the linkage between puberulence and defective seed coat, the defective seed coat trait must be regulated by a much more complex genetic system than a simple recessive gene. In all previously reported cases, the defective seed coat trait was inherited identically to the simple recessive p2. This would indicate that if these traits are in fact controlled by separate genes, defective seed coat would be a simple recessive. However, smooth seeds were not seen in the M1 families that produced apparently smooth seeds (Table 2).

It is possible that several genes are involved in the expression of defective seed coat. Lui (1949) conducted a genetic study of all known defective seed coat traits, and found that the physiological expression of defective seed coat is controlled by a series of several genes including genes for seed coat color, pubescence color, and at least four genes for defectiveness. However, Lui's genetic analysis was flawed. In the case of T31, he based his research on studies done by Woodworth and Williams (1938) which were disproved. In addition, the conclusions he drew about the defective trait in T31 were based on non-T31 plants, which he incorrectly assumed had the same genetic control as T31 (Williams, unpub.).

It is very likely that environmental conditions play a role in the expression of defective seed coat. Further research is necessary to ascertain whether the linkage between puberulence and defective seed coat in T31 has been broken, and if so, what type of genetic system controls its expression. All plants were grown isolated in the greenhouse to prevent natural cross pollination, which necessitated extreme overcrowding of the plants. This resulted in very few seeds from each plant, and many of the seeds were quite small. Because so few seeds were produced, an accurate genetic ratio for expression of the smooth seed characteristic could not be established. The progeny of the M4 plants that did not display the defective seed coat should

Table 1. Pedigree of M4 plants without defective seed coat trait.

M1 plant no.	M2 plant no.	M3 plant no.	M4 plant no.
1	22	2	2
1	22	14	2
1	22	14	3
1	22	14	4
129	778	6	9
129	778	11	14
183	1405	8	19
184	1404	4	4
184	1404	4	7
184	1422	7	1
184	1422	7	3
184	1422	7	11
184	1422	7	14
184	1422	7	16
184	1422	12	5
184	1422	12	10
184	1422	12	11
184	1422	12	20
184	1432	3	1
184	1432	3	7
184	1432	3	14
184	1432	3	17
184	1432	6	7
184	1432	10	10
229	1609	5	11
229	1609	10	3
341	1957	12	1
341	1957	15	31
490	2173	1	24
490	2173	3	4
490	2177	5	16
490	2177	5	24

Table 2. Ratio of M2 and M3 generations producing smooth seeds from seven segregating M1 families of nitrosomethyl urea (NMU)-treated seed.

M1 plant no.	Ratio of M2 plants without de trait	Ratio of M3 plants without de trait
1	2/35#	2/30#
129	4/38	2/60
183	8/47	1/120
184	3/24	6/45
229	1/15	2/15
341	1/15	2/15
490	1/15	3/15

#Ratio of smooth seeded plants to total number of plants for families that continue to segregate.

be grown under normal field conditions to determine whether defective seed coat is expressed under those conditions. These plants should also be used in crossing experiments to determine whether this defective seed coat trait is identical to any other defective seed coat traits found in G. max.

Conclusion: Mutagenesis with nitrosomethyl urea was used to attempt to break the linkage between defective seed coat and puberulence in T31. Thirty two M4 puberulent plants with smooth seed coats were obtained. However, it is not certain whether seeds with non-defective seeds are the result of a mutation, or merely environmental influence on the expression of this trait. In any case, further research should be completed in order to establish whether or not a mutation has taken place, and if so, to determine the genetic system regulating this trait.

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R.D.R. Culbertson

J.E. Harper

T. Hymowitz

4) A quick method of resolving Soybean Kunitz trypsin inhibitor using polyacrylamide gel electrophoresis.

Introduction: Soybean Kunitz trypsin inhibitor (KTI) is a globular, water soluble protein consisting of 181 amino acid residues with a molecular weight of 21,384 daltons. It is present in soybean seed in four allelic forms: Ti^a , Ti^b , Ti^c , and ti in which Ti^a , Ti^b , and Ti^c are inherited codominantly and the ti (null) is inherited as a recessive allele (Orf and Hymowitz, 1979).

Orf and Hymowitz (1979) used 10% polyacrylamide disc electrophoresis based on the Davis gel and buffer system (Davis, 1964) to resolve various alleles of KTI. This method was later modified by Prischmann and Hymowitz (1988) using 1.5 x 140 x 140 mm continuous resolving slab gels instead of disc (tube) gels. This procedure takes 4-5 hours of gel running time.

In this paper we present a procedure whereby KTI can be resolved on a mini-gel system in a greatly reduced time period. In this procedure we used 10% non-denaturing discontinuous polyacrylamide slab gel electrophoresis based on Laemmli's gel and buffer conditions without sodium dodecylsulfate (SDS), 2-mercaptoethanol, and heating of samples (Laemmli, 1970). These conditions are briefly as follows:

Sample Preparation: Using a razor blade, a chip of approximately 5 mg was taken from a soybean seed (cut opposite to the hilum to save seed for germination). This was ground in 200 μ l of buffer containing 0.092 M Tris-Cl pH 6.8, 0.023 M $CaCl_2$, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF, 100 mM stock made in absolute ethanol). Alternately, one-half of the seed was ground in 3 ml of buffer. These samples were then centrifuged to remove the undissolved residue and 5 μ l of the supernatant was mixed with 5 μ l of sample loading buffer containing 20% glycerol and 0.005% bromophenol blue before applying to gel (DO NOT heat the samples). The samples were kept cold (4°C) during the extraction procedure and used immediately following the extraction or stored in -20°C for later analysis.

Gel and Buffer System: We used Mini-PROTEAN II Dual Slab Cell unit from BioRad Laboratories, Richmond, CA for polyacrylamide gel electrophoresis. However, other brand mini-gel units could also be used. The gel and buffer system is based on Laemmli's procedure (1970) without SDS. In brief, the resolving gel consisted of 10% acrylamide: N,N'-methylene-bisacrylamide

(30:0.8), 0.375 M Tris-Cl pH 8.8 in distilled water, and the stacking gel consisted of 5% acrylamide : N,N'-methylene-bisacrylamide (30:0.8), 0.125 M Tris-Cl pH 6.8 in distilled water. The gels were polymerized by adding ammonium persulfate and N,N,N',N'-tetramethylethylenediamine (TEMED) to the final concentration of 0.05 and 0.1%, respectively. The running buffer consisted of 0.025 M Tris base and 0.192 M glycine (pH not adjusted, the natural pH will be about 8.3). The gels were cast with 0.75 mm spacers (gel dimensions 0.75 x 70 x 80 mm) and the stacking gel was about 1 cm long from the bottom of the wells to the resolving gel. The gels were run at a constant power of 200 volts for 30 min. Two gels could be run per unit at a time and up to 30 samples were analyzed by using 15-well combs. By having an extra set of gel casting apparatus one can run several gels in a relatively short period of time. Detailed description of gel casting and sample loading procedures are given in the instruction manual.

Staining Procedure: After the run, gels were stained in a large petri dish containing 50 ml coomassie blue stain (0.15% Coomassie Brilliant Blue R-250 in methanol : acetic acid : distilled water in proportion of 5:1:4, respectively) for 15 min with gentle shaking. Destaining was done for 30-45 min with 2-3 changes of destaining solution (methanol : acetic acid : distilled water in proportion of 3:1:6, respectively). These gels were read on a fluorescent light background immediately or stored in 7% acetic acid solution for later use.

Identification of KTI: Ti^a, Ti^b, and Ti^c alleles of KTI were identified by their migration pattern with the R_f values of 0.66, 0.62, and 0.70, respectively (in this gel system) or by using appropriate standards (for example: Amsoy 71 - Ti^a; Jefferson - Ti^b; PI 196172 - Ti^c). Purified Soybean KTI (from Sigma Chemical Co., St. Louis MO) was also often used (0.2-0.5 ug/lane) as a standard for KTI-band location. The ti (null) allele was identified by the absence of KTI band at the above mentioned three positions.

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Krishna P. Kollipara
Juliann M. Domagalski
Theodore Hymowitz

5) Response of Williams isolines to eight races of Phytophthora
megasperma f.sp. glycinea.

Phytophthora root and stem rot of soybean is caused by the fungus Phytophthora megasperma f.sp. glycinea (Pmg). Resistance to various races of Pmg is conferred by seven genes designated Rps1 to Rps7, with multiple alleles at Rps1 and Rps3. The resistant phenotype is always dominant. Since the identified Rps genes are not from closely related cultivars, investigations that compare race responses to different Rps genes are hampered by variation due to other genetic differences among the cultivars. Isolines of Harosoy and Williams that contain different Rps genes have been developed by backcrossing and should eliminate much of this variation. Pmg responses of Harosoy isolines were reported by Buzzell et al. (1987); however, Harosoy itself was shown to be resistant to some races. A desirable feature of the Williams isolines is that the recurrent parent Williams has been reported to be susceptible to all known races of Pmg.

The reactions of the isolines to the races of Pmg provide useful information for breeders and pathologists since they may be presumed to be single-gene effects. Buzzell et al. (1987) reported that the reactions of the Harosoy isolines to 19 races of Pmg were consistent with the reaction of the donor parents to the same races, but apparently inconsistent for four other

Table 1. Response of Williams isolines and differential standards to races of *Phytophthora megasperma* f.sp. *glycinea*.

Cultivar ¹	Rps gene	Donor parent ²	Pmg race ³										
			1	2	3	7	9	16	20	27			
Isolines													
Williams	<u>Rps</u>		S	S	S	S	S*	S	S	S	S	S	S
L75-6141	<u>Rps1</u>	Union (Mukden)	R	R	S	S	S	R	S	R	S	R	R
L77-1863	<u>Rps1-b</u>	Harrell	R	S	R	R	R	R	S	S	S	S	S
L75-3735	<u>Rps1-c</u>	Lee 68 (Arksoy)	R	R	R	R	R	R	S	S	S	S	S
L77-1794	<u>Rps1-k</u>	Kingwa	R	R	R	R	R	R	S	S	S	S	S
L76-1988	<u>Rps2</u>	D54-2437 (CNS)	R	R	R	R	S*	R*	R	R	R	R	R
L83-570	<u>Rps3</u>	PI86.972-1	R	R	R	R	S	R	R	R	S	S	S
L85-2352	<u>Rps4</u>	PI86.050	R	R	R	R	S*	S*	R	R	R	S	S
L85-3059	<u>Rps5</u>	PI91.160	R	R	-	S	R	-	S	-	S	-	-
Differential cultivars													
Harosoy	<u>RpsH</u>	Mandarin [Ot.] (Mandarin)	S	S	S	S	S	S	R	S	S	S	S
Harosoy 63	<u>RpsH Rps1</u>	Blackhawk (Mukden)	R	R	S	S	S	S	R	S	S	R	R
Sanga	<u>Rps1-b</u>		R	S	R	R	R	R	S	S	S	S	S
PI103091	<u>Rps1-d</u>		R	R	R	R	R	R	R	R	R	R	R
PI171442	<u>Rps3</u>		R	R	R	R	S	R	R	S	S	S	S
PI86972-1	<u>Rps3</u>		R	-	R	S	R	R	R	-	-	-	-
PI86050	<u>Rps4</u>		R	R	R	S	S	R	-	R	-	-	-
L62-904	<u>Rps5</u>		R	R	R	S	R	R	-	R	-	-	-
Altona	<u>Rps6</u>	PI194.654	R	R	R	S	S*	R	R	R	R	R	S

¹ L-strains are isolines of Williams. Data for PI86050 and L62-904 are from Athow 1985.

² Ancestral source of Rps genes are in parenthesis.

³ Asterisk indicates less than 100% of the plants inoculated were susceptible (S) or resistant (R).

racess which were not reported. We are investigating the reaction of the Williams isolines, their donor parents, and other cultivars with the same Rps genes to 27 races of Pmg. The reaction of nine Williams isolines and seven differential cultivars to eight races of Pmg is reported in this paper.

Materials and Methods: Soybean seeds were obtained from the USDA soybean germplasm collection at the University of Illinois. The L-strains are isolines developed here at Urbana by backcrossing to Williams (BC5 or BC6) with selection for the specified Rps gene. Cultivars used in the differential set or as donor parents of the Rps gene were included as controls to ensure the identity of the race and the Rps gene.

Hypocotyls of cultivars were inoculated with races 1, 2, 4, 5, 6, 7, 9, 10, 13, and 14. Taproots of cultivars grown in aeroponic culture were inoculated with races 3, 16, and 27.

Results and Discussion: The reactions of the 16 tested cultivars to eight races of Pmg are listed in Table 1. The cultivars PI 86,050 (Rps4) and L62-904 (Rps5) were not tested but included in the table for comparative purposes. Data for these two cultivars are from Athow (1985). Seven other supposed races were used in these tests but were not pathogenic on any of the cultivars. The resistance to the races conferred by each Rps gene was consistent with the literature (Athow, 1985). Cultivars with different ancestral sources of the same Rps gene (Rps1-b, from L77-1863 and Sanga; Rps3 from L83-570 and PI 171,442) responded similarly when challenged by the same race.

The isolines L76-1988 (Rps2) and L85-2352 (Rps4) expressed variation in response to infection by races 7 and 9. Williams (rps) and Altona (Rps6) expressed variation in response to race 9. Plants were scored as susceptible or resistant based on the rating system of Moots et al. (1983), who also reported variation in the response of certain cultivars to certain races of Pmg. However, a plant expressing an intermediate level of resistance (R*) is not necessarily resistant. Wagner (1989) reported that, while only 30% of the plants of the cultivar Altona were killed following hypocotyl inoculation with Pmg 27 (an R* score), 100% of the plants grown in aeroponic culture were rated susceptible following taproot inoculation with Pmg 27.

Variable and intermediate Pmg responses have been attributed to genetic

and environmental effects (Buzzell et al., 1982; Wagner, 1989). The response of L77-1863 and L85-2352 to races 7 and 9 could indicate a genetic background (Williams) effect on the performance of these Rps genes when challenged by a particular race or isolate of Pmg. The more extensive investigation we are currently conducting on the reaction of the isolines, donor parent, and ancestral source should provide more insight into the sources of variation in the Pmg:soybean interaction,

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R. E. Wagner

R. L. Bernard

6) Disease response of soybean cultivars to *Phytophthora megasperma* f.sp. *glycinea* race 16 following gene transfer at the *Rps1* locus.

Phytophthora root and stem rot of soybean is caused by the fungus *Phytophthora megasperma* f.sp. *glycinea* (Pmg). Resistance is conferred by single dominant genes located at seven loci, designated *Rps1* to *Rps7*. Multiple alleles have been identified at the *Rps1* and *Rps3* loci. Extensive breeding efforts to control Pmg with *Rps* genes have resulted in the development and commercial release of numerous backcross-derived cultivars. However, no gene or cultivar provides resistance to all of the 27 reported races of Pmg (Wagner, 1989). The purpose of this research was to investigate the changes in response to race 16 as a result of gene transfer at the *Rps1* locus.

Materials and Methods: Thirty one backcross-derived cultivars (including six unreleased lines) and their recurrent parents were obtained from the USDA soybean germplasm collection or, in the case of HARO 15XX, from R.I. Buzzell, Agriculture Canada, Harrow. Seeds were planted in a greenhouse sandbench. Seven days after planting, the hypocotyls were inoculated with mycelia and were covered with wet cheesecloth for 12 hours. Mycelia were grown in sterile 1/5 X lima bean broth and originated from zoospores. Cultures were used within one week of establishment.

Results and Discussion: The results for 31 cases of allele substitution at the *Rps1* locus are presented in Table 1 for three races of Pmg. The cultivars are grouped into categories based on similarity of gene change and disease response. Data on races 1 and 3, which were the races used in most of this variety development, are based on our results or in some cases are data published with the release of the variety.

For race 1, 27 of the gene substitutions converted susceptible cultivars to resistant ones (*rps1* to *Rps1*, *Rps1-c*, or *Rps1-k*) whereas the remaining four were resistant and remained so (*Rps1* to *Rps1-c* or *Rps1-k*). For race 3, 11 of the gene transfers left the variety susceptible (*rps1* to *Rps1*) whereas the remaining 20 changed susceptibility to resistance (*rps1* or *Rps1* to *Rps1-c* or *Rps1-k*). For race 16, which was not the object of selection in any of these gene transfers, the results were more complex: In eight transfers susceptibility remained; in four transfers resistance remained; in eight transfers susceptible cultivars were made resistant, but in 11 transfers

Table 1. Change in reaction to Phytophthora megasperma f.sp. glycinea in backcross-derived cultivars with gene transfer at the Rps1 locus.

Backcross-derived cultivar*	Gene change (source)	Change in disease response#		
		<u>Race 1</u>	<u>Race 3</u>	<u>Race 16</u>
1. Amsoy 71, Chippewa 64, Clark 63, Cutler 71, Lindarin 63, Union (Williams BC4), Weber 84, L65-4049, (Wayne BC5)	<u>rps1--Rps1</u> (Mukden)	S--R	S--S	S--R
2. Williams 79, L79-1380 (Clark BC5)	<u>rps1--Rps1-c</u> (Arksoy)	S--R	S--R	S--S
3. Elgin 87, Gnome 85, Hobbit 87, Sprite 87, Williams 82, L77-2015 (Clark BC5)	<u>rps1--Rps1-k</u> (Kingwa)	S--R	S--R	S--S
4. Beeson 80, Wells II	<u>Rps1--Rps1-c</u> (Arksoy)	R--R	S--R	R--S
5. Century 84, Pella 86	<u>Rps1--Rps1-k</u> (Kingwa)	R--R	S--R	R--S
6. Harosoy 63, Hawkeye 63, Hodgson 78	<u>rps1--Rps1</u> (Mukden)	S--R	S--S	R [^] -R
7. Corsoy 79, Vickey (Corsoy BC4)	<u>rps1--Rps1-c</u> (Arksoy)	S--R	S--R	R [^] -S
8. Haroson (Hodgson BC6) Vinton 81, L85-129 (Harosoy BC5)	<u>rps1--Rps1-c</u> (Higan)	S--R	S--R	R [^] -S
9. Harper 87, L78-189 (Corsoy BC7)	<u>rps1--Rps1-k</u> (Kingwa)	S--R	S--R	R [^] -R [^]
10. HARO 15XX	<u>rps1--Rps1-k</u> (Kingwa)	S--R	S--R	R [^] -R [^]

*Where the original recurrent parent is not used in the variety name it is given in parentheses.

#S=susceptible; R=resistant.

[^]Resistance to race 16 may be explained by a linked gene (Rps?) as proposed by Buzzell et al. (1987).

resistant cultivars became susceptible. This change in disease response from resistant to susceptible for 11 of the 31 gene substitutions underscores the limitation of individual Rps genes in providing long-term control of Pmg as well as the problems with a breeding strategy that focuses on replacing alleles at a single locus.

The eight backcross-derived cultivars in Table 1 categories 7 to 10 were derived from five recurrent parents: Harosoy plus 3 cultivar with Harosoy as an ancestor plus the unknown Harper. All five recurrent parents are susceptible to races 1 and 3 and resistant to race 16 suggesting that all carry the gene "Rps?" proposed by Buzzell et al. (1987) and Anderson and Buzzell (1988). The eight backcross-derived cultivars of categories 7 to 10 all received alleles that confer susceptibility to race 16 (Rps1-c or Rps1-k). Seven of them became susceptible to race 16, indicating that "Rps?" was lost, therefore suggesting that "Rps?" is allelic to Rps1. However, the eighth derived cultivar HARO 15XX (category 10) remained resistant to race 16, confirming the observation and interpretation of Buzzell et al. (1987) that "Rps?" is at a separate but closely linked locus. More evidence for two loci is present in the data of Moots et al. (1983) where Harosoy 63, with the gene Rps1 transferred from 'Mukden', retained Harosoy's resistance to race 12, to which Mukden was susceptible. It appears that both Harosoy 63 and HARO 15XX are crossover types with genotypes Rps1 Rps? and Rps1-k Rps?, respectively.

If this two-locus explanation is correct, then only the four changes to race 16 susceptibility of categories 4 and 5 are due to the specified gene substitution. The seven changes to susceptibility of categories 7 to 9 were caused by linkage.

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Richard E. Wagner

Richard L. Bernard

7) Ancestry of US/Canadian commercial cultivars developed by public institutions.

Most US and Canadian commercial cultivars are traceable through documented pedigrees to their imported ancestral strains. This pedigree history provides an interesting overall picture of the origins of the North American commercial soybean crop, and, when considered in detail, may provide useful guidance information to the breeder concerning potential new combinations of germplasm. In this work we considered only the 321 cultivars developed by selection from controlled crossing at public institutions; however, since the cultivars from private companies are mostly derived from public ancestors, the overall picture for private cultivars would be similar.

These tables provide the percentage of relationship, or in other words, the theoretical probability (in %) of a single allele from an ancestor occurring in a specific public soybean cultivar. For example, in an A x (B x C) cross, the A ancestor would be 50% and the B and C ancestors would be 25% each. A rather small group of introductions constitutes the ancestry of the domestic crop. In the following tables, the 17 ancestors that appear in more than 25 descendant grain-type cultivars are represented in individual columns and the remaining 66 ancestral cultivars that appear in 1 to 22 descendant cultivars are specified in the last column. In many cases, different sublines from a single introduction were used [such as 'Mandarin' and 'Mandarin (Ottawa)' from PI 36,653 or 'AK (Harrow)', 'Illini', and 'Sl00' from 'AK']. For simplicity we have lumped these sublines together in one column and treated them as one ancestor. Also provided for each domestic cultivar are maturity group, year of release and total number of ancestors that the cultivar has descended from.

The cultivars have been split into two groups according to major uses. The first group is "Grain Types" in which the soybeans are used mainly for oil and meal production. The second group is "Specialty Types" which are primarily intended for use as vegetables or in traditional Asian foods. Summaries at the bottom of each table provide information on the probable percent of genetic material that each ancestor provided to the total of the listed cultivars and the number of cultivars that trace to the 17 major

ancestors. For "Grain Types" this is further divided into those adapted to three different latitude regions.

These tables should be helpful in several ways. They can be used in choosing parents for crosses that would have a higher probability of combining different alleles. Another use is in finding cultivars that have a common ancestor (e.g., an ancestor that carries a disease-resistant allele such as 'Kingwa'). These tables also allow one to determine if any recorded relationship exists between cultivars and to quantify that relationship.

Information in these tables were derived from "Origins and Pedigrees of Public Soybean Varieties in the United States and Canada" (1988) or directly from the releasing institution for more recent releases. Other summaries of pedigrees have been done by Delannay et al. (1983) and Allen and Bhardwaj (1987).

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David G. Lohnes

R. L. Bernard

Table 1. Ancestry (in %) of Domestic Grain Type Cultivars

Cultivar	Mauiity Group	Yr Released	# Ancestors	Habertland	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksay	Mukden	PI 54610	Richland	PI 70128	CNS	Palmetto	Roanoke	L37-1355	China	No 171	% Other Ancestors
Origin				Korea	China	China	China	China	China	China	Korea	China	China	China	China	China	China	China	Japan	China		
Introduced				1901	1902	1906	1911	1912	1913	1913	1913	1914	1920	1921	1926	1926	1927	1927	1929	1931		
Maturity				VI	VII	IV	II,III	III,V	O,I	III	VI	II	I	II	IV	VII	VII	VII	III			
Acadian	VIII 43	2																				50% P160106, 50% FC04910
Acme	OO 53	2							50.00				12.50									50% Manitoba Brown
Ada	OO 72	6						18.75	31.25					12.50						12.50		12.5% Manitoba Brown
Adams	III 48	2						50.00		50.00												
Adelphia	III 64	6					12.50	25.00	12.50	25.00												
Alamo	IX 78	3						37.50								37.50						25% P1240664
Akoma	OO 66	2																				50% Flambeau, 50% P1194654
Amor	II 79	6						37.45	37.50	12.45		0.049		0.049						12.50		
Amor 89*	II 89	9						37.06	37.33	12.45		0.048		0.29		0.097				12.30		0.012% Kingwa
Amscy	II 65	3						37.50	37.50	25.00												
Amscy 71*	II 70	5						37.40	37.50	24.90		0.098		0.098								
Anoka	I 70	4					18.75		18.75					12.50								50% Korean
Archer*	II 90	13					15.03	12.50	33.78	6.25		6.06	6.05	11.51		1.59						.39% Flambeau & P1194654, .38% P184946, .012% Kingwa
Armedo	V1 45	2																				50% Mammoth Yellow, 50% Laredo
Avery	IV 87	12		2.73	7.23	2.73							7.23	7.03		13.87	2.73					6.25% P188788
Baas	III 89	12					17.69	12.50	17.69	5.86		.0015	1.56	10.77	6.25	18.70		6.25	6.25			0.78% P184946
Bay	V 78	9		3.13	8.59		3.13	9.38		18.75	15.63		8.59			17.19		15.63				
Bedford	V 77	9		5.47	8.20	5.47		24.61		5.47			8.20			24.61	5.47					12.5% P188788
Benson	II 68	7					12.50	6.25	31.25			12.50	12.50	12.50								
Benson 80*	II 79	8					12.45	6.23	31.13		0.39	12.45	12.45	12.45								
Bell	I 89	10					19.53	8.59	26.56	6.25		3.13	3.91	12.50		3.13						12.5% P188788
Bethel	IV 61	3													25.00				25.00			50% FC33243
Bicentennial	OO 83	5						12.45	37.35			0.098		0.098								50% P1360955
Bienville	VIII 58	5											25.00									25% P160406, 12.5% Ocotan, 12.5% Biko
Blackhawk	I 50	2										50.00		50.00								
Bonus	IV 71	7					6.25	12.50	43.75			12.50	6.25	12.50								
Bossier	VIII 58	2						50.00								50.00						
Bradley	V1 83	10						27.88		5.91	0.44		8.50			27.88	5.66					6.25% P188788
Bragg	VII 63	5						25.00					18.75			25.00	12.50					
Braxton	VII 79	6						23.44					19.11			25.00	11.72					1.56% Biko

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Maturity Group	# Released	Haberlandt	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksoy	Mukden	PI 54610	Richland	PI 70128	CNS	Palmetto	Roanoke	L37-1355	No. 171	% Other Ancestors
Brim	VI	90 12	3.13	7.23			16.41		2.34	7.03		7.23		7.81	21.48	10.16	7.81			6.25% FC31745, 3.13% PI171442
Bryan	VI	90 10	5.86	8.50	3.13		27.88		5.91	0.44		8.50			27.88	5.66				6.25% PI88788
BSR 101	I	85 10		6.25		15.09	12.50	33.84	6.25		6.25	6.25	11.63		1.54					0.39% PI84946
BSR 201	II	82 10		3.13		20.80	10.94	34.86			3.13	3.13	13.87		3.13				6.25	0.78% PI84946
BSR 301	III	79 6				35.37		35.37			.0031		21.53		6.16					1.56% PI84946
BSR 302	III	80 9		6.25		23.94	3.13	33.31			6.25	6.25	17.02		3.08					0.78% PI84946
Burlison	II	88 12	2.34	7.81		7.81	13.28	31.25	2.34		6.25	7.81	6.25		5.47					6.25% FC31745, 3.13% PI171442
Calland	III	68 7		12.50		12.50	6.25	31.25			12.50	12.50	12.50							
Capital	O	44 2				50.00													50.00	
Carter	III	86 7				19.92	9.38	19.92	9.38				11.72		4.69					25% PI88788
Celest	V	77 5				6.25		6.25						12.50	31.15	5.86				50% PI80837, 25% FC33243
Centennial	VI	76 9	6.25	8.79	0.78		31.15		6.35	0.88										
Century	II	79 7		9.38		9.38	37.50				12.50	9.38	12.50							
Century 84*	II	84 10		9.08		9.91	9.47	37.15	0.39		12.11	9.08	12.59		0.19					0.024% Kingwa
Chamberlain	III	86 10		6.25		26.42	4.69	31.11	3.13		3.13	6.25	15.54		3.10					0.39% PI84946
Chapman	II	90 14	.0034	.0034	0.010	16.05	20.17	34.58	7.67	.0061		.0034	8.90		5.48	.0046			6.25	0.78% PI82263, 0.098% Korean
Chico	OO	83 8				2.34	26.56	16.41			9.38		10.94		6.25				15.63	12.5% PI317335
Chief	IV	40 2				50.00	50.00													
Chippewa	I	54 3				37.50		37.50				25.00								
Chippewa 64*	I	64 4				37.35		37.35			0.20		25.10							
Clark	IV	53 3				37.50		37.50					25.00							
Clark 63*	IV	63 5				37.11		37.11			0.39		25.00		0.39					
Clay	O	68 5				18.75	25.00	18.75					12.50						25.00	
CN210	II	83 9		6.25	0.76	18.62	9.18	27.99			6.30	6.25	18.59		6.06					
CN290	II	83 9		6.25	0.76	18.62	9.18	27.99			6.30	6.25	18.59		6.06					
Cobb	VIII	73 9		20.70			1.56					20.70			7.81	11.72	12.50			12.5% PI68406, 6.25% Ootolan, 6.25% Biloxi
Coles	I	76 7				4.69	9.38	42.19			12.50		18.75							6.25% Jagan, 6.25% Kanro
Colquitt	VII	89 6		14.26			30.47					14.26			31.25	8.98				0.78% Biloxi
Columbus	IV	71 5		12.50		31.25		31.25				12.50	12.50							25% Mantocha Brown
Comet	O	53 2						75.00												6.25% PI91110
Conrad	II	88 13		3.91		12.11	14.84	23.83	4.69		3.13	3.91	8.59	3.13	9.38		3.13			6.25% PI88788, 3.13% PI90763, 3.13% PI171442
Cordell	V	89 11	11.33	4.10	5.08		24.41		11.33			4.10			24.41	2.73				
Corsoy	II	67 3					37.50	37.50											25.00	

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Maturity Group	Yr Released	# Ancestors	Haberlandt	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksoy	Mukden	PI 54610	Richland	PI 70128	CNS	Palmetto	Roanoke	L37-135S	No 171	% Other Ancestors
Coraxy 79*	II	79	5					37.68	36.91		0.024					0.77				24.61	
Crawford	IV	77	8		6.25		28.91	6.25	28.91	6.25			6.25	14.06		3.13					
Crest	OO	57	2						75.00												25% Manitoba Brown
Crockett	VIII	88	6		12.50			12.50								12.50					50% PI171451, 6.25% Mammoth Yellow, 6.25% Laredo
Cumberland	III	78	7				13.28	25.00	32.03	6.25				7.81		3.13				12.50	
Curtis	VI	58	2					50.00								50.00					
Custer	IV	67	7			3.03	12.12	24.23	12.12			0.024		24.26		24.23					
Cutler	IV	68	5		12.50		31.25		31.25				12.50	12.50							
Cutler 71	IV	71	7		13.27		30.86		30.86			0.012	13.27	11.72		.0061					
Dare	V	65	7	12.50	12.50			12.50		12.50		6.25	12.50			12.50		25.00		12.50	12.5% Flambeau, 12.5% PI194654
Daniel	O	86	8				4.69	18.75	23.44				18.75	9.38		12.50		25.00		12.50	12.5% PI180501
Davis	VI	65	5		18.75						25.00			9.38						12.50	50% FC33243
Dawson	O	83	7				4.69	21.88	32.81			6.25									
Delmar	IV	63	4				12.50		12.50						25.00						
Delaxy 4500	IV	89	12	3.13	4.69	3.13	6.64	26.56	16.02	6.25			4.69	3.91		15.63	3.13			6.25	
Delaxy 4900	IV	89	13		1.66	2.64	6.79	30.22	6.79	3.13	1.56		1.66	4.20	3.13	30.81		4.30	3.13		
DeSoto	IV	79	8		6.25		28.91	6.25	28.91	6.25			6.25	14.06		3.13					
Dorman	V	52	2							50.00	50.00										
Douglas	IV	80	9		6.25		19.53	9.38	28.91	6.25		6.25	6.25	14.06		3.13					50% PI200492
Dowling	VIII	78	6		6.25			12.50			12.50		6.25			12.50					25% Seneca
Dunn	I	69	4				31.25		31.25					12.50							
Durocrop	VIII	81	8		15.63		15.63		15.63		12.50		15.63	6.25		6.25		12.50			
Dyer	V	67	5	12.50		12.50		31.25		12.50						31.25					
Edison	III	90	11		1.56		14.84	14.84	31.25	6.25		1.56		9.38		3.13				3.13	12.5% Elgin
Egyptian	IV	84	15	2.73	4.10	3.49	12.28	18.36	12.28	2.73		0.055	4.10	12.27		18.41	2.73				6.25% PI88788, 0.0010% T145, 0.0010% T201
Elf	III	77	13	1.56	3.52		13.28	15.63	13.28	6.25	1.56		3.52	7.81	9.38	13.28		1.56	9.38		
Elgin	II	84	?																		from the population AP6 [see Crop Science 15:739]
Elgin 87	II	87	?				0.41	0.19	0.41	0.19				0.24		0.097					98.44% Elgin, 0.012% Kingwa
Epps	V	83	12	3.91	5.27	3.32		31.57		3.98	0.66		5.27			31.57	2.73	2.34			6.25% PI88788, 3.13% PI96983
Essex	V	72	7		3.13			25.00					3.13		12.50	31.25		12.50	12.50		
Evans	O	74	5					25.00	37.50			12.50		12.50						12.50	
Fayette	III	81	7				19.92	9.38	19.92	9.38				11.72		4.69					25% PI88788
Flyer	IV	88	12		1.46		14.10	18.35	14.10	6.63			1.46	8.29	5.86	17.97		5.86	5.86		0.049% Kingwa

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Maturity Group	Yr Released	# Ancestors	Heberlandt	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Aksoy	Murken	PI 54610	Richland	PI 70128	CNS	Palmetto	Roanoke	L37-1355	No 171	% Other Ancestors
Ford	III	58	3				25.00	28.13	25.00	6.25			9.38	50.00		28.13	6.25				1.56% PI60406, 0.78% Oootan, 0.78% Blosi
Forres	V	72	8	6.25	9.38	6.25		26.32		5.52	0.41	0.11	10.50			27.10	6.74	1.56			0.20% T145, 0.20% T201
Foster	VIII	81	13	5.47	10.50	2.73										12.21					
Franklin	IV	77	9			1.51	24.56	12.12	24.56	18.70		0.049		7.86		3.13					
Fremont	III	85	7				13.28	24.95	32.03				6.25			25.00		25.00			25% FC31745
Gail	VI	78	6		6.25			12.50					15.63			25.00	6.25	25.00			
Gazy 17	VII	77	6		15.63			12.50													
Gibson	IV	42	2							50.00											50% Midweg
Glenwood	O	87	8				4.69	20.31	37.50	3.13		6.25		9.38					6.25		12.5% PI248404
Gnome	II	79	13	1.56	3.52		13.28	15.63	13.28	6.25	1.56		3.52	7.81	9.38	13.28		1.56	9.38		
Gnome 85*	II	85	14	1.54	3.46		13.49	15.57	13.49	6.35	1.54		3.46	7.93	9.23	13.17		1.54	9.23		0.012% Kingwa
Gordon	VII	84	9	3.13	4.69	3.91		38.18		3.22	0.88		4.69			38.18	3.13				
Govan	VII	77	6		15.63		25.00			12.50			15.63			25.00	6.25				
GR883*	III	87	12		1.46		14.10	18.35	14.10	6.63			1.46	8.29	5.86	17.97		5.86	5.86		0.049% Kingwa
GR893*	III	88	12		1.46		14.10	18.35	14.10	6.63			1.46	8.29	5.86	17.97		5.86	5.86		0.049% Kingwa
Grant	O	55	3				25.00		25.00												50% Santea
Gregg	VII	83	8		9.38	0.78		36.62		0.098	0.88		9.38			36.62	6.25				
Hack	II	84	9		3.13		18.37	14.04	32.43	9.35		3.15	3.13	11.77		4.64					
Hagood	VII	90	12	3.13	9.86	0.39		21.83		3.17	6.69		9.86		3.13	26.51	2.93	9.38	3.13		
Hamilton	IV	89	14	0.78	1.76		6.64	26.63	25.10	3.13	0.79		1.76	3.91	4.69	7.03		0.78	4.69	12.30	
Harcor	II	75	5					34.35	46.80			0.049		0.049						18.75	
Hardee	VIII	62	7		6.25								6.25			12.50		25.00			25% PI60406, 12.5% Oootan, 12.5% Blosi
Hardin	I	80	9		1.66		3.86	32.81	36.67			.0015	1.66	1.47		.0008			21.88		
Hardome	O	53	2					25.00	75.00												
Hark	I	66	4					12.50	37.50			25.00		25.00							
Harbin	I	74	4					12.45	37.35			25.10		25.10							
Harly	I	48	2					50.00	50.00												
Haroson*	I	87	8					18.68	28.12					6.29		0.024				12.45	24.80% PI180501, 0.20% Iligan
Haroszy	II	51	2					25.00	75.00					0.20							
Haroszy 63*	II	63	4					24.90	74.71					0.20							
Harper	III	84	?																		from an unknown dialled-cross population
Harper 87*	III	87	?					0.41	0.19	0.41				0.24		0.097					98.44% Harper, 0.012% Kingwa
Harwood	II	70	5					9.38	12.45	71.73		0.098		6.35							

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Maize Group	Yr Released	# Ancestors	Haberlandt	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksoy	Mukden	PI 54610	Richland	PI 70128	CNS	Palmerto	Roanoke	L37-1355	No 171	% Other Ancestors
Hawkeye	II	47	2									50.00		50.00							
Hawkeye 63*	II	63	2							12.43		50.00		50.00						6.25	0.39% Kingwa
Hayes	III	89	9				13.18	24.93	31.93	25.00		0.024		7.78		3.10					
Henry	II	60	3					25.00		25.00				50.00							
Hill	V	59	4	25.00				25.00		25.00						25.00					
Hobbs	III	81	13	1.56	3.52		13.28	15.63	13.28	6.25	1.56		3.52	7.81	9.38	13.28		1.56	9.38		
Hobbs 87*	III	87	14	1.54	3.46		13.49	15.57	13.49	6.35	1.54		3.46	7.93	9.23	13.17		1.54	9.23		0.012% Kingwa
Hodgson	I	74	6				9.38	18.75	28.13				6.25	6.25						12.50	25% PI180501
Hodgson 78*	I	78	7				9.30	18.80	27.91			0.20		6.40						12.60	24.80% PI180501
Hood	VI	58	4		12.50								12.50			25.00		50.00			
Hood 75*	VI	75	5		12.45						0.39		12.45			24.90		49.80			
Howard	VIII	90	14	5.47	9.35	4.10		25.46		5.49	0.22		9.35			25.85	6.10	0.78			6.25% PI88788, 78% PI60406, .39% Ocootán & Bixia
Hoyt	II	86	15	0.78	1.76		6.64	24.99	30.04	3.13	0.78	0.024	1.76	3.93	4.69	6.64		0.78	4.69	9.38	
Hurdston	V	87	10		3.13			12.50		6.25	6.25		3.13		6.25	18.75		12.50	6.25		25% PI71506
Hutton	VIII	72	6		9.38			12.50					9.38			37.50	6.25	25.00			
Improved Pelican	VIII	50	3																		50% PI60406, 25% Ocootán, 25% Bixia
Jack	II	89	11		0.83		11.89	21.09	28.30	4.69		.0008	0.83	6.59		2.34				10.94	12.5% PI88788
Jackson	VII	53	3		37.50								37.50				25.00				
James	V	75	6		12.50		18.75		18.75				12.50		12.50						25% FC33243
Jeff	VI	81	12	3.32	6.23	3.32		32.52		3.37	2.49		6.23			33.54	3.13	2.05			3.13% PI88788, 0.68% FC33243
Johnson	VIII	83	12	0.78	12.70		17.19				0.78		6.45		4.69	30.08	3.13	13.28	4.69		3.13% Mammoth Yellow, 3.13% Laredo
Jupiter	IX	71	3					25.00								25.00					50% PI24664
Jupiter-R*	IX	82	3					25.00								25.00					50% PI24664
Kaola	I	90	14		0.014	0.041	7.31	29.43	30.16		0.024	1.56	0.014	6.41		0.41		0.018		21.48	3.13% Korean, 0.0031% PI86024
Karo	I	89	9		4.69		5.86	17.97	36.33			9.38	4.69	10.16						7.81	3.13% PI180501
Keller*	II	83	10		12.35		12.35	6.18	30.88		0.39	12.35	12.35	12.35							0.39% PI86972, 0.39% PI54615
Kent	II	61	4		25.00		25.00		25.00				25.00								
Kenwood	II	89	7				10.92	4.70	15.57			0.049		6.29		3.13				3.15	50% Eigen, 6.20% PI180501
Kershaw	VI	82	5		15.63						12.50		15.63			18.75		37.50			
Kino	VI	66	5				18.75	25.00	18.75					12.50		25.00					
Kirby	VIII	83	13	5.47	10.50	2.73		26.32		5.52	0.44		10.50			27.10	6.74	1.56			1.56% PI60406, 0.78% Ocootán, 0.78% Bixia
Kuntz*	III	89	8				25.94	12.21	25.94	12.21				15.26		6.10					1.56% PI157440, 0.77% Kingwa
Lakota	I	81	7																		from the population AP6 [see Crop Science 15:739]

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Yr released	Maturity Group	# Ancestors	Haberland	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksoy	Mukden	PI 54610	Richland	PI 70128	CNS	Palmetto	Hoanoke	L37-1355	No. 171	% Other Ancestors
Lamar	VI 89	12		6.25	5.32	0.20		23.41		6.27	1.78		5.32			23.41	3.03				12.5% FC31745, 6.25% PI171442, 6.25% PI229358
Lawrence	IV 81	9			6.25		19.53	9.38	28.91	6.25		6.25	6.25	14.06		3.13					
Lee	VI 54	2						50.00								50.00					
Lee 68*	VI 68	3						49.22			1.56					49.22					
Lee 74*	VI 74	4						48.83			0.78					48.83					1.56% FC33243
Lefbre	VI 84	10		5.86	8.50	3.13		27.88		5.91	0.44		8.50			27.88	5.66				6.25% PI88788
Lincoln	III 43	2					50.00		50.00												
Lindarn	II 58	2					25.00		75.00												
Lindarn 63*	II 64	3					24.90		74.71			0.39									
Linford	III 89	8					23.14	10.89	23.14	10.89						5.44					12.5% PI88788, 0.39% Kingwa
Lloyd	VI 88	14		3.32	4.69	2.47	0.26	36.54	0.26	3.37	0.44		4.69	0.51		36.54	3.13				3.12% PI88788, 0.68% FC33243
LN83-2356	IV 88	15		0.39	3.22		8.20	18.75	19.92	3.13	0.39	4.69	3.22	8.59	5.47	11.91			3.52	5.47	3.13
Logan	III 85	9			3.13		11.73	20.31	35.17	12.50		3.13	3.13	7.84		3.08					
Mack	V 71	10			1.76	5.27	0.29	41.70	0.29		3.13		1.76	0.59		42.87		2.34			
Madison	II 60	3					25.00		50.00			25.00									
Maple Amber	OO 81	7						6.23	18.68			0.049		0.049							50% PI438477, 12.5% Flambeau, 12.5% PI194654
Maple Arrow	OO 76	5						12.45	37.35			0.098		0.098							50% PI438477
Maple Donovan	O 86	6						23.40	42.08			0.073		0.073						9.38	25% PI438477
Maple Glen	OO 87	9					8.59	14.06	30.47	3.13				4.69		3.13				6.25	25% PI438477, 4.69% Manitoba Brown
Maple Isle	OO 84	6						18.46	55.37												25% PI194641, 59% T122, 29% PI88351 & Rokusun
Maple Presto	OO 79	5						9.38	25.00	6.25											50% PI438477, 9.38% Manitoba Brown
Maple Ridge	OO 84	6						12.50	18.75			6.25		6.25						6.25	50% PI196491
Marcus	II 89	9					18.54	15.64	32.56	1.56		0.049		10.58		5.47				9.40	6.20% PI180501
McCall	OO 78	6					9.38	6.25	40.63			12.50		18.75							12.5% Manitoba Brown
Mead	III 81	8			3.13		20.31	6.25	39.06				3.13	15.63		6.25					
Merit	O 59	4						25.00				25.00		25.00						25.00	
Miami*	II 84	10			6.18		6.18	12.35	43.24		0.39	12.35	6.18	12.35							0.39% PI86072, 0.39% PI54615
Miles	IV 78	6					22.27	18.75	22.27					14.84		18.75					3.13% PI84631
Minnato	O 89	6						12.50	18.75			6.25		6.25						6.25	50% PI437267
Monroe	I 48	2							50.00			50.00									
Morgan	IV 86	10					24.54	15.43	24.54	6.05				15.28		12.59					1.56% PI84631, 0.0008% Kanto, 0.0002% T145 & T201
Morsey	OO 70	4							25.00					25.00							25% Manitoba Brown, 25% Seneca
Narow	V 84	11			13.38	2.64	0.15	27.10	0.15		7.81		13.38	0.29		27.69	6.25	1.17			

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Yr Released	Group	# Ancestors	Haberlandt	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksoy	Mukden	PI 54610	Richard	PI 70128	CNS	Palmetto	Roanoke	L37-1355	No 171	% Other Ancestors
Nathan	V	80	9	5.47	8.20	5.47		24.61		5.47			8.20	12.50		24.61	5.47				12.5% PI88788
Nebsey	II	79	7		12.50		12.50	6.25	31.25			12.50		12.50							50% Mammoth Yellow, 50% Oskotan
Nela	VIII	45	2																		0.20% PI84946
Newton	II	90	11		6.25	0.36	16.86	10.84	30.92	3.13		6.28	6.25	15.11		3.80					50% Flambeau
Nordchief	O	54	3									25.00		25.00							25% Manitoba Brown
Norman	OO	69	3					12.50	62.50												
OAC Arles	O	86	10				6.84	4.69	27.15			9.38		13.67		0.78				6.25	12.5% Flambeau & 194.654, 6.25% Manitoba Brown & T260
OAC Libra	O	85	6					18.73	37.43			6.30		6.30							25% PI360955
OAC Musca	O	87	7				4.69	9.35	38.90			6.30		9.42							25% PI360955, 6.25% Manitoba Brown
OAC Pines	O	85	10				6.84	4.69	27.15			9.38		13.67		0.78					12.5% Flambeau & 194.654, 6.25% Manitoba Brown & T260
OAC Scorpion	OO	86	7				4.69	9.35	38.90			6.30		9.42							25% PI360955, 6.25% Manitoba Brown
Oakland	III	78	9		6.25		19.53	9.38	28.91	6.25		6.25	6.25	14.06		3.13					
Ogden	VI	40	2		50.00					50.00											
Oksey*	IV	71	6				12.30	24.61	12.30			0.78		25.39		24.61					
Ozzie	O	83	7				4.69	21.88	32.81			6.25		9.38						12.50	12.5% PI180501
Padre	IX	88	11	7.42	7.03	4.69		25.39		7.42			7.03			25.39	4.69				6.25% PI159925, 3.13% FC31745, 1.56% PI171442
Pagoda	OO	39	2						50.00												50% Manitoba Brown
Pella	III	79	9		6.25		19.53	9.38	28.91	6.25		6.25	6.25	14.06		3.13					
Pella 86*	III	86	10		6.05		19.74	9.47	28.83	6.44		6.05	6.05	14.11		3.22					0.024% Kingwa
Pennyrite	IV	87	11		1.56		13.28	18.75	13.28	6.25			1.56	7.81	6.25	18.75		6.25			
Perrin	VII	88	8		20.51			24.22					14.26			25.00	8.98				3.13% Mammoth Yellow, 3.13% Laredo, 0.78% Biko
Perry	IV	52	2												50.00						
Perrish	IV	84	10	9.38	1.56			21.88		9.38			1.56		6.25	25.00		6.25			12.5% PI171450
Pharrah	IV	89	12	6.25	4.69	3.13	3.13	23.44	3.13	12.50	6.25		4.69	6.25		23.44	3.13				
Pickett	VI	65	5			3.13		48.05		0.39	0.39					48.05					
Pickett 71*	VI	71	5			1.56		48.24		0.20	1.76					48.24					
Pixie	IV	80	13	1.56	3.52		13.28	15.63	13.28	6.25	1.56					13.28		1.56			
Plate	II	82	8		6.23		6.23	31.15	24.98	24.90		0.24	6.23	0.049							
Pomona	IV	74	5		12.50		12.50	12.50	50.00				12.50								
Portage	OO	64	2						62.50												37.5% Manitoba Brown
Preston	II	85	13		3.13		25.40	7.03	32.43	4.69		0.004				4.63					6.25% PI91110, 0.0092% Kanro, 0.0031% T145 & T201
Protana	II	69	6		12.50		12.50		12.50			25.00									25% PI65368
Proto	O	89	8				9.36	3.13	18.73			0.021		6.36							25% PI261475, 25% PI189880, 12.5% Pridesay II

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Maturity Group	# Ancestors	Häberlandt	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksoy	Mukden	PI 54610	Richland	PI 70128	CNS	Palmetto	Roanoke	L37-1355	No 171	% Other Ancestors
Provar	II	69	4			18.75	12.50	56.25					12.50							6.25% PI88788, 0.098% T145, 0.98% T201
Pyramid	IV	85	15	2.73	3.49	12.28	18.36	12.28	2.73		0.055	4.10	12.27		18.41	2.73				
Rampage	I	69	3			37.50		37.50					25.00					18.75		
Ransom	VII	70	9	3.13	7.03		18.75			3.13		7.03		18.75	20.31		3.13			
RCAT Alliance	I	87	10		3.11	16.39	12.48	35.07	6.25	0.20	6.23	3.11	14.04		3.13					25% PI360955, 25% PI297518
RCAT Persian	I	89	7				12.50	28.13			3.13		3.13						3.13	.20% 86972 & 84637, .0015% Kanro, .00038% T145 & T201
Regal*	IV	86	11			26.70	12.06	26.70	12.06				15.66		6.41					
Renville	I	52	3			37.50		37.50					25.00							
Renit	III	87	12		1.46	14.10	18.35	14.10	6.63			1.46	8.29	5.86	17.97		5.86	5.86		0.049% Kingwa
Rhodes	V	90	15	5.86	5.86	22.46			5.08	0.78		5.86		4.69	22.85	2.73	0.78	4.69		6.25% PI88788, 6.25% FC31745, 3.13% PI171442
Rillito	V	74	5			18.75	25.00	18.75	6.25	6.25			12.50		25.00					
Ripley	IV	85	13		1.56	4.69	9.38	14.06	6.25			1.56	3.13		3.13		6.25		6.25	25% PI71506, 12.5% PI180501
Ross	III	60	3			25.00		50.00			25.00		25.00							
Scott	IV	59	5			12.50	25.00	12.50				12.50			25.00					
Semmes	VII	65	5	12.50			25.00		25.00						25.00					
Sharkey	VI	87	11	7.81	4.39	0.39	26.51		7.86	0.44		4.39			26.51	2.93				12.5% FC31745, 6.25% PI171442
Shelby	III	58	3			37.50		37.50					25.00							
Sherman	III	85	9		3.13	18.36	14.06	32.42	9.38		3.13	3.13	11.72		4.69					
Shore	V	74	5		6.25							6.25			12.50		25.00			
Sibley	I	86	7																	50% PI8837
Simpson	O	82	7			4.69	18.75	32.81			9.38		12.50						9.38	12.5% PI180501
Shan	II	78	8			4.69	15.63	32.81			12.50		15.63						6.25	12.5% PI180501
Sohoma	VI	78	6		9.38	17.97	15.63	27.34	6.25				10.94		3.13		12.50		6.25	12.5% Korean
Sparks	IV	81	9		6.25	19.53	9.38	28.91	6.25	13.28		9.38					12.50			
Spencer	IV	88	12		5.47	14.84	10.94	32.03	6.25		6.25	6.25	14.06		3.13					
Spride	III	80	13	1.56	3.52	13.28	15.63	13.28	6.25	1.56		5.47	9.38	3.13	1.56			1.56		3.13% FC33243
Spride 87*	III	87	14	1.55	3.49	13.38	15.60	13.38	6.30	1.55		3.52	7.81	9.38	13.28		1.56	9.38		
Stafford	IV	86	11		1.56	12.50	6.25	12.50	6.25	18.75		3.49	7.87	9.30	13.23		1.55	9.30		0.0061% Kingwa
Steele	I	72	4									1.56	12.50		9.38		6.25	12.50		
Stonewall	VII	88	15			12.50	37.50		25.00				25.00							
Sturdy	I	89	9	5.08	2.93		24.88		4.32	1.00		9.35		4.69	25.66	4.93	1.56	4.69		0.78% PI60406, 0.39% Orootan, 0.39% Rhoxa
Swift	O	72	6			5.86	17.97	36.33			9.38	4.69	10.16						7.81	3.13% PI180501
Tennessee Non Pop	VII	42	2		50.00	18.75	12.50	18.75				50.00	12.50						12.50	25% Korean

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Maturity Group	Yr Released	# Ancestors	Habersland	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksoy	Mukden	Pl 54610	Richard	Pl 70128	CNS	Palmerito	Roanoke	L37-1355	No 171	% Other Ancestors
Thomas	VII	88	10	3.13	13.96	0.39		27.29		3.17	0.44		13.96			28.08	8.79				0.78% Bix'd
TN 4-86	IV	86	12	2.73	7.23	2.73	14.45	15.43	14.45	5.86			7.23	7.03		13.87	2.73				6.25% P188788
TN 5-85	V	85	11	3.13	6.25	3.13		26.56		3.13			6.25		6.25	29.69	3.13	6.25	6.25		
Toano	V	85	13	3.13	1.56		1.17	18.75	1.17	3.13			1.56	3.91	6.25	21.88		6.25	6.25		25% P180837
Tracy	VI	73	6	9.38				21.88		9.38						21.88					25% FC31745, 12.5% P1171442
Tracy-M*	VI	79	6	9.38				21.88		9.38						21.88					25% FC31745, 12.5% P1171442
Traverse	O	65	2				25.00		75.00												
Twigg	VI	87	9	6.25	9.08	3.52		29.64		6.30	0.44		9.08			29.64	6.05				
Union*	IV	77	9				26.81	12.11	26.81	12.11				15.73		6.44					0.002% Kano, 0.0004% T145, 0.0004% T201
Vanoy	O	70	4				12.50		12.50												50% Ilabaro, 25% Flambeau
Vickery*	II	78	12		0.027	0.082	0.55	36.98	36.88		0.049		0.027	0.33		0.82		0.037	24.22		0.006% P186024
Viking	III	42	2				50.00	50.00													
Volante	VII	42	2		50.00								50.00								
Wabash	IV	48	2				50.00			50.00											
Wakers	V	90	13	3.13	11.38	4.44	0.073	27.61	0.073	3.13	3.91		11.38	0.15		27.91	6.25	0.59			
Ware	IV	78	8	6.25			2.34	12.50	2.34	6.25				7.81		12.50					50% P180037
Wayne	III	64	4				34.38		34.38					18.75		12.50					
Weber	I	79	9		3.13		12.50	12.50	31.25			6.25	3.13	12.50					6.25		12.5% Korean
Weber 84*	I	85	9		3.32		12.40	12.40	31.45			6.45	3.32	12.50					6.05		12.11% Korean
Wells	II	72	7		6.25		6.25	12.50	43.75			12.50	6.25	12.50							
Wells II*	II	78	8		6.23		6.23	12.45	43.58		0.39	12.45	6.23	12.45							
Wilkin	O	72	5					25.00	37.50			12.50		12.50					12.50		
Will*	III	79	7				26.72	12.30	26.72	12.30				15.77		6.15					0.002% T117
Williams	III	71	6				26.56	12.50	26.56	12.50				15.63		6.25					
Williams 79*	III	79	7				26.15	13.07	26.15	12.30	0.024			15.38		6.92					
Williams 82*	III	81	7				26.35	12.40	26.35	12.40				15.50		6.20					0.78% Kingwa
Winchester*	III	84	8				26.35	12.40	26.35	12.40				15.50		6.20					0.39% P186972, 0.39% 84.637
Wirth	I	69	3				37.50		37.50					25.00							
Woodworth	III	74	6				26.56	12.50	26.56	12.50				15.63		6.25					

* Derived by 4 or more backcrosses or by reselection.

Table 1. (Cont'd)

Cultivar	Group	Maturity	Yr Released	# Ancestors	Haberlandt	Tokyo	Peking	Manchu	AK	Mandarin	Durfield	Arksoy	Mukden	PI 54610	Richland	PI 70128	CNS	Palmetto	Roanoke	L37-1355	No. 171	% Other Ancestors
Wright	VII	79	5			9.38											37.50	6.25				12.5% FC33243
Wye	IV	71	10			3.13		23.44	6.25	17.19	12.50			3.13	3.13	12.50				6.25		
York	V	67	6			6.25					25.00	25.00		6.25			12.50		25.00			
Young	VI	84	8			10.94						12.50		10.94		6.25	21.88		18.75	6.25		
Zane	III	84	10			3.13		16.41	17.19	30.47	6.25		3.13	3.13	10.94		3.13				6.25	
41 Group 000-0 cultivars#						0.00	0.00	4.19	12.41	33.81	0.30	0.00	4.70	0.00	7.06	0.00	0.26	0.00	0.00	0.00	5.95	31.31% other ancestors
number of descendants						0	0	18	31	38	3	0	24	0	28	0	4	0	0	0	18	21 ancestors in 35 cultivars
160 Group I-IV cultivars#						0.33	2.86	15.68	13.94	27.85	5.12	0.31	4.24	2.86	11.46	1.58	4.80	0.11	0.50	1.46	2.15	4.59% other ancestors
number of descendants						20	84	137	128	148	82	25	81	84	136	25	92	6	25	25	31	32 ancestors in 69 cultivars
87 Group V-X cultivars						2.48	9.17	1.19	20.67	0.92	3.54	3.14	0.00	8.89	0.42	1.22	23.63	2.75	5.72	0.93	0.00	14.36% other ancestors
number of descendants						35	65	11	67	10	39	42	0	64	8	13	75	38	34	11	0	19 ancestors in 44 cultivars
All 288 cultivars#						0.93	4.34	0.46	15.74	20.61	3.94	1.12	3.03	4.26	7.50	1.24	9.81	0.89	2.00	1.09	2.05	11.41% other ancestors
number of descendants						55	149	46	166	226	124	67	105	148	172	38	171	44	59	36	49	60 ancestors in 148 cultivars

* Totals do not include cultivars with unknown pedigrees [Elgin (II), Elgin 87 (II), Harper (III), Harper 87 (III), Kenwood (II), and Lakota (I)]

* Derived by 4 or more backcrosses or by reselection.

Table 2. Ancestry (in %) of Domestic Specialty Type Cultivars

Cultivar		Maturity Group	Yr Released	# Ancestors	Habermann	Tokyo	Peking	Manchu	AK	Mandarin	Dunfield	Arksoy	Mukden	PI 54610	Richland	PI 70128	CNS	Palmetto	Roanoke	L37-1355	No. 171	% Other Ancestors	
Origin					Korea	Japan	China	China	China	China	China	Korea	China	China	China	China	China	China	China	Japan	China		
Introduced			1901		1901	1902	1906			1911	1912	1913	1913	1914	1920	1921	1926	1927	1927	1929	1931		
Maturity			VI		VI	VII	IV	II,III	III,V	O,I	III	VI	II	I	I	II	IV	VII	VII	III			
Camp*	V	89	8			1.56			12.50						1.56	6.25	15.63	6.25	6.25			50% unknown wild soybean	
Disoy	I	67	4							25.00												25% Jogun, 25% Kanro	
Emerald	IV	75	6			6.25				6.25					25.00							50% Aoda, 25% Hidaio	
Grande	O	76	6							34.38					6.25							25% Korean, 12.5% Jogun, 12.5% Kanro	
HP201	I	88	12			0.83				4.20	21.14	39.36	6.06	9.08	0.83	9.81	0.004				10.94	3.03% Jogun, 3.03% Kanro, 0.78% Higan	
HP202	I	88	10							7.70	11.79	40.52	9.08	9.08	15.97	15.97	1.56				3.13	4.54% Jogun, 4.54% Kanro, 1.17% Higan	
HP203	I	88	10							7.70	11.79	40.52	9.08	9.08	15.97	15.97	1.56				3.13	4.54% Jogun, 4.54% Kanro, 1.17% Higan	
HP204	I	88	12			0.41				4.37	15.31	40.70	9.08	9.08	13.99		0.002				5.47	4.54% Jogun, 4.54% Kanro, 1.17% Higan	
IL1	II	89	14			1.76				6.64	7.81	6.64			1.76	3.91	6.64					50% T208	
IL2	III	89	14			0.78	1.76			6.64	7.81	6.64			1.76	3.91	6.64					50% PI408026	
Kahala	IV	69	4										12.50		12.50							50% Bansei, 25% FC33243	
Kaikoo	IV	69	4										12.50		12.50							50% Bansei, 25% FC33243	
Kailua	IV	69	4										12.50		12.50							50% Bansei, 25% FC33243	
Kanrich	III	56	2												25.00							75% Kanro	
Kim	III	56	2												25.00							75% Sac	
Magna	II	67	3							50.00												25% Jogun, 25% Kanro	
Marion	II	76	7							4.69	21.88	42.19	12.50		6.25							6.25% Jogun, 6.25% Kanro	
Merrimax	I	86	4																			50% A100, 12.5% Jogun, 12.5% Kanro	
Mokapu Sumer	IV	69	4										12.50		12.50							50% Bansei, 25% FC 33243	
Prize	II	67	3							50.00												25% Jogun, 25% Kanro	
SS201	II	89	5										10.94		10.94				21.88			43.75% PI257435, 12.5% PI81762	
SS202	II	89	6							16.41	5.47	32.81	10.94		21.88							12.5% PI135624	
Vance	V	86	8			1.56				12.50				1.56		6.25	15.63		6.25			50% unknown wild soybean	
Verde	III	67	3																			50% Aoda, 25% Jogun	
Vinton	I	78	7							4.69	9.38	42.19	12.50		18.75							6.25% Jogun, 6.25% Kanro	
Vinton 81*	I	81	8							4.54	9.47	42.04	12.11		18.16							6.05% Jogun, 6.05% Kanro, 15.6% Higan	
% of all cultivars					0.06	0.54	0.00			3.20	5.65	20.16	0.72	0.06	4.99	0.54	11.38	0.84	1.83	0.84	0.87		46.92% other ancestors
# of cultivars tracing to					2	7	0	12	12	16	3	2	12	7	20	4	8	0	0	1.38	0.84	4	16 ancestors in 26 cultivars

* Derived by 4 or more backcrosses or by reselection.

ASGROW SEED COMPANY
PO Box 210
Marion AR 72364

1) Variable expression of seedcoat permeability.

The impermeable seedcoat trait has been shown to protect seed quality from deterioration due to weathering associated with late-season rains and delayed harvest (Hartwig and Potts, 1987; Degago and Longer, 1989). A disadvantage of impermeability in improved cultivars is the need for scarification of planting seeds to obtain a productive stand (Kilen, 1989; Moore et al., 1989). Kilen and Hartwig (1978) suggested that the impermeable trait may be controlled by as few as three genes which would imply expression of intermediate levels of permeability (Tinius et al., 1988). Thus, there may be a level of impermeability that would protect seed quality yet be sufficiently low, or degrade in storage, such that scarification would not be required. The practical use of such a level would depend on its repeatability under diverse environmental conditions. The objective of this experiment was to measure the variability in expression of different levels of seedcoat permeability across environments.

Plants for this study originated as selections from Kirby*(Forrest(3)*D77-12480) crossed with two sibling males, F81-5588 or F81-5590, which donated the impermeable trait. From F_6 hill plots grown at Raleigh, NC in 1986, ten single plants were selected, threshed mechanically, and tested by placing 25 seeds from each plot in a petri dish that contained a germination paper and deionized water. The percentage of seeds still impermeable at 24 h was used to select among sibling plants for different levels of permeability. The procedure was repeated in 1987 with plants from F_7 hills grown at Hartsville, SC, and three sets of near-isolines (each set tracing to a single F_6 plant) were identified. The lines were grown in single rows at Raleigh and Hartsville in 1988, and at Hartsville, Gainesville and Quincy FL, Alexandria LA, and Stoneville MS, in 1989. Ten single plants were harvested from each row at the Raleigh and Florida locations, while other locations were harvested in bulk by row. Plants were threshed mechanically except those grown at Alexandria, which were threshed manually to minimize scarification. Rows harvested in bulk were tested for permeability on the

basis of 100-seed samples, whereas single plant data are from 25-seed samples.

Lines in SET 1 showed a wide range in permeability levels across environments (Table 1). HSC2 ranged from 10% impermeable seeds at Hartsville in 1989 to 79% at Hartsville in 1988. Likewise, HSC3 ranged from 2 to 27% at the same location in different years. Complete permeability of seeds from HSC4 was consistent across all seven environments. While the repeatability of levels was poor across environments, rankings of the three lines within environments was very consistent. Thus, the environmental effect on expression of permeability levels was more important than the genetic effect. Data from single plants suggest that HSC3 is intermediate in level to HSC2 and HSC4, and may have an additional gene for the trait compared to the other two lines.

Two lines in SET 2, HSC22 and HSC24, had nearly consistent low levels of impermeability across environments. Standard errors do not support a significant difference between them, although HSC24 sometimes had higher levels, especially at Alexandria, where seeds were presumably not scarified in threshing. Based on single plant data, HSC25 is apparently still segregating for a single gene and is likely composed of types similar to both HSC22 and HSC23. Again, there was low repeatability of levels across environments for some lines, while ranking of lines was consistent. Additionally, the range in percentage of impermeable seeds was largest at the Hartsville location.

Lines in SET 3 had responses similar to lines in SETS 1 and 2. HSC54 has a low level of impermeability across environments, while HSC53 was intermediate. Single plant data also suggest that HSC53 is segregating for a single gene that conditions two levels of permeability. There was also some variability across environments for expression of trait in SET 3, but, like the other sets, the ranking of genotypes within the environments was consistent.

The consistency of ranking among lines across the seven environments suggest that CxE interaction was not an important factor in the low repeatability of permeability levels. The strong environmental effect has important implications on the practical use of seedcoat impermeability in improved cultivars. A line with an intermediate level may fail to express that level in some environments and thus not provide the protective function that is desired. This can occur even at the same location in different years (e.g., Hartsville in 1988 and 1989). A line with a relatively low level may express

a significantly higher level in some environments, necessitating an added scarification treatment. Furthermore, lines that express a moderate to high level, requiring scarification for planting, may be resisted by farmers who intend to use harvested seed for planting the following year. Such a cultivar would likely need to be superior in some important aspect, or significantly lower in purchase price, in order to provide farmers with sufficient incentive to use it.

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Table 1. Percentage of impermeable soybean seeds after 24 hr soak.

		1988		1989				
	Line	NC#	SC	GV	QU	MS	SC	LA
-----#-----								
SET 1	HSC2	22±6	79	62±5	19±3	33	10	46
	HSC3	10±5	27	13±6	4±3	18	3	10
	HSC4	0	0	0	0	0	0	0
SET 2	HSC22	0	0	1±1	0	0	0	0
	HSC23	82±4	88	88±3	63±4	66	24	80
	HSC24	8±8	7	1±1	2±2	3	0	22
	HSC25	18±6	36	38±13	22±9	25	7	46
SET 3	HSC53	31±13	54	39±14	38±10	36	26	62
	HSC54	0	0	4±22	4±4	4	0	10

NC - Raleigh
 SC - Hartsville
 GV - Gainesville

QU - Quincy
 MS - Stoneville
 LA - Alexandria.

Christopher Tinius

WILLIAM PATERSON COLLEGE

Biology Department

Wayne NJ 07470

and

THE UPJOHN COMPANY

Molecular Biology Division

Kalamazoo MI 49001

and

DNA PLANT TECHNOLOGY CORPORATION

Cinnaminson NJ 08077

1) Intrinsic GUS-like activities in soybean.

The beta-glucuronidase (GUS) gene of E. coli has been developed as a gene-fusion marker for higher plants by Jefferson et al. (1986). The objective of this report is to document and alert workers in soybean research about the presence of intrinsic GUS-like activity in cultivated soybean and its wild relative soja.

Seeds and plants of soybean cv. 'Williams 82' and 'Pella' and the wild relative Glycine soja Sieb. & Zucc. PI 65,549 were tested. Tissues were surface disinfected and tested under aseptic conditions with histochemical and qualitative fluorometric procedures.

Experimentation and Results: Histochemical GUS Assay -- Tissues from different seedlings, mature plants, young and mature seeds of each soybean cultivar and mature seeds and seedlings of soja were tested. Leaves were surface disinfected by dipping in 70% ethanol for 1 min. After the ethanol evaporated, the leaves were cut into 1-2 mm wide strips in a laminar flow hood. Fruits and seeds were surface disinfected by soaking in 10% Clorox for 10 min followed by rinsing with three changes of sterile distilled water in a laminar flow hood. Fruits and seeds were dissected and part of the fruit wall, seed, and embryo were cut into thin sections.

A modified Jefferson's (1987) procedure was followed. The testing solution contained 2 mM substrate, X-Glu, in a pH 7.0 phosphate buffer (29.3 mM K_2HPO_4 + 20.7 mM KH_2PO_4) in which 10 mM EDTA and oxidation catalysts of 0.5 mM potassium ferricyanide and 0.5 mM potassium ferrocyanide were added. The sterile tissues were incubated with filter-sterilized testing solution overnight in a 37°C dark

incubator. The chlorophylls, if present, were cleared by soaking the tissue in 95% ethanol before data were taken.

The seedling and mature plant leaves of both tested soybean cultivars and the seedling leaves of soja expressed negative intrinsic GUS-like reactions with histochemical tests.

The immature soybean fruits of various developmental stages showed strong histochemical staining reactions in all four tested parts: fruit walls, seed coats, endosperms and embryos. Positive reactions were also found in the cotyledons, plumules, hypocotyls, and radicles of mature seeds of soybean cultivars and soja. Usually, the vascular bundles expressed stronger staining activities than the surrounding tissues in both young and mature tissues.

Changes in the pattern of intrinsic GUS-like activities during seed germination -- Sterile seeds of soybean cv. 'Williams 82' were germinated under aseptic conditions. Histochemical GUS tests were performed on the seed sections or germinated seedlings at the 0, 2, 4, 6, 8, 10, 12, and 14th day of germination.

The intrinsic GUS-like activities existed in every part of mature dry seeds of soybean. The intensities of staining increased during the early period of the germination process, then dropped rapidly after the fourth day of germination. Practically no detectable positive staining reactions were found on any part of the seedlings by the tenth day of germination (Table 1).

Changes in intrinsic GUS-like activities of immature embryos during in vitro culture -- Immature pods of soybean cv. Pella were harvested and surface-disinfected. Embryos, ranging from heart to early cotyledon stages, were excised and cultured in modified B5 liquid medium (Hu and Sussex, 1986). Histochemical GUS tests were performed at the 0, 1, 2, 3, 5, and 7th day of culturing.

Immature embryos expressed strong GUS-like staining activities at dissection. All the staining activities of smaller embryos and most of the staining activities of larger embryos disappeared after one to two days of in vitro culture. By the fifth and seventh days, only small blue spots infrequently appeared on certain larger embryos.

Qualitative fluorometric GUS assay -- Seeds of soybean cv. Pella, at various developmental stages (0.5, 1, 2, 3, 4, 5, and 6 mm in length plus mature dry seeds) were tested along with leaves of corn (negative controls) and E. coli GUS gene containing transgenic tobacco (positive controls).

Tissue extract in MUG-containing lysis buffer was incubated at 37°C for 0

and 24 h for soybean embryos. The blue fluorescence was observed visually with a long-wave UV light box.

Soybean seeds expressed positive reactions at all tested developmental stages, but the intensities were not as strong as those of transgenic tobacco leaves (the positive control). The negative controls, the sweet corn leaves, showed no activity.

ELISA GUS assay -- Standard ELISA procedure was carried out to assay the presence of *E. coli* GUS in soybean tissue with a dilution series of purified *E. coli* GUS as the positive control. Immature seeds of 3, 5, 7, and 12 mm length were sampled. The detection level of the assay was as low as 7.2 ng.

Anti-GUS activity was detected in all of the positive control wells. No anti-GUS activity was detected in any of the test samples assayed.

Table 1. Change in intrinsic GUS-like activities via histochemical test during soybean cv. Williams 82 seed germination.

Days under germination conditions	Plant parts			
	Plumule	Cotyledon	Hypocotyl	Radicle or tap root
0	++	++	++	++
2	++	++	++	++
4	+++	+++	+++	+++
6	-	++	+	-
8	-	+	-	-
10	-	-	-	-
12	-	-	-	-

Discussion: Fifty-two plant species, covering some gymnosperms and all the key groups of angiosperms, were chosen by us for surveying their intrinsic GUS-like activities (Hu et al., 1990). Histochemical (overnight incubation) and qualitative fluorometric (24 h incubation) assays indicated that, with few exceptions, such activities were detected in certain part(s) of the fruit walls, seed coats, endosperms or, especially, the embryos of the tested plants. Only 11 species showed such activities (usually quite weak) in the vegetative organs of seedlings/mature plants. The expressing of intrinsic GUS-like activities in various plant species were also reported by Wenzler et al. (1989), Plegt and Bino

(1989), and Alwen et al. (1990).

The intrinsic GUS-like activities in the seeds diminished during soybean germination process. The same phenomenon was also observed in the germinating string bean seeds (Hu et al., 1990). This might be the reason that Jefferson et al. (1987) did not detect such activities in the vegetative organs of the species he tested. It is possible that these activities may not completely disappear even at the mature phase of plant life (see the previous paragraph). High level of activities reappear at the onset of the reproductive phase, as demonstrated during anther (Plegt and Bino, 1990), fruit and seed development.

In vitro culture conditions quickly turned off most of the intrinsic GUS-like activities in excised immature soybean embryos. But our work on histochemical GUS assay of various types of cultured soybean tissues indicated that in vitro culture conditions did not always turn off such activities (unpublished data). These data suggested that the microenvironment around the cell played an important role in the expression of gene(s) for such activities.

Since the ELISA tests were negative, it appears that the protein(s) responsible for the intrinsic GUS-like activity in the soybean seeds and other plant tissues is not antigenically similar to the E. coli GUS enzyme. Alwen et al. (1990) found that about 50-fold higher concentration of saccharic acid 1,4-lactone is required to inhibit the plant GUS activity, compared to the E. coli GUS. They also found the pH optima is 5.0 for plant GUS and close to neutral for the bacterial enzyme. We found that in soybean the histochemical staining patterns carried out at pH 7 are general and dispersed with weak intensities. The true transgenic GUS staining patterns, on the other hand, have well-defined area of transformed cells and highly intense colorations (Hu et al., 1990). Therefore, it should be possible to assay the E. coli GUS in transgenic soybean plants.

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Ching-yeh Hu
Wm. Paterson College

Paula P. Chee
The Upjohn Company

Paul D. Miller
DNA Plant Technology Corporation

WILLIAM PATERSON COLLEGE

Biology Department

Wayne NJ 07470

and

SOYBEAN RESEARCH INSTITUTE

Heilongjiang Academy of Agricultural Science

Harbin, The People's Republic of China

1) Embryoids from soybean anther culture.

Embryo-like structures have been obtained from calli derived from soybean anther culture. Immature floral buds of 2.5 - 3.5 mm length, with sepal length 1/3 to 1/2 of bud length, were harvested from field-grown soybean cv. 'Williams 82' and Asgrow A1929.

Buds were pre-incubated under 4°C for 8 days, 7-8°C for 4 or 8 days, 37°C for one day, or no pre-incubation. Anthers were dissected and cultured on a medium of B5 salts, enriched with 16 organic compounds, 0.5 mg/l BAP, 2 mg/l 2,4-D, 9% sucrose and solidified with 0.3% agarose. From 120 to 480 anthers were used in each pre-incubation treatment per cultivar. Calli appearing during the first three months of incubation were mostly derived from anther walls and were removed with the anthers transferred onto fresh medium. One month later, five of the newly grown calli from each of the 4 and 7 - 8°C pre-treatments of cv. Williams 82, and three from 4°C pre-treatment of cv. A1929 developed embryo-like structures (embryoids). One callus produced more than 10 well-defined embryoids from globular to early cotyledon stages. Most of the embryoids expressed various degrees of abnormalities. Some of the embryoids were transferred to "maturation" or "multiplication" media. The well-known recalcitrant nature of soybean tissues beyond early seedling stage makes those embryoids not likely derived from somatic tissue. They were presumably the products of microspores via "androgenesis".

X. J. Zhuang

C. Y. Hu

Y. Chen

Wm. Paterson College

G. C. Yin

Heilongjiang Acad. Agric. Sci.

UNIVERSITY OF MARYLAND EASTERN SHORE
Princess Anne MD 21853

1) Screening vegetable soybean for corn earworm resistance.

Corn earworm (Heliothis zea-Boddie) is a highly prolific serious polyphagous pest of soybean, with a cosmopolitan distribution. It is known as a major soybean pest capable of causing serious economic losses from damage done to foliage and pods. If control measures are not taken in time, pod feeding could go undetected, resulting in serious crop loss (Turnipseed, 1985). Immature pods attacked by this pest either drop from the plant or fail to develop properly (Scott et al., 1983). Developing seeds are eaten in their pods, thus reducing yield. Boldt et al. (1975) and Smith and Bass (1972) reported that each corn earworm larva can destroy up to eight pods. If the larval population is highly dense during the early developmental stage of the beans, chemical treatment may become necessary in order to save the crop. Larval population level is most likely to exceed economic thresholds in warmer months, especially when soybean is planted in close proximity to maize.

As people worldwide become cholesterol conscious, they will consume more soybean foods. As farmers strive to meet such demand economically and remain in business, the need for combatting pests capable of inflicting economic losses is inevitable. Chemical control measures have thus become the most popular method of insect control. However, the increasing and indiscriminate use of chemicals has drawn much concern and criticism from consumers and environmentalists. The basis of such concerns hinges on the hazardous effect of chemicals on the environment and pesticide residue build-up in food. According to a 1988 study conducted by the Food Marketing Institute, 75% of consumers believe that pesticide residues in food are a serious health hazard. If such negative concerns are allowed to prevail, farmers will face a tough battle in selling their produce. In order to eliminate the danger from chemicals, it is necessary for researchers to develop cultivars resistant to pests of economic importance. It is therefore the objective of this study to identify vegetable soybean germplasm resistant to corn earworm pod damage.

Materials and Methods: Ninety five vegetable soybean cultivars belonging to maturity groups III - V were planted in May, 1990. Forty six of these are new cultivars incorporated in the ongoing study this year. Each cultivar was planted in a 2.5-meter single row plot with row spacing of 60 cm. In each

maturity group, one row of commercial cultivar identical in maturity group was used to separate experimental plots and form borders. There were four replications arranged in randomized complete block design. Plants were harvested at ground level between October and November. Three randomly selected plants from each plot represented the sample. The number of pods damaged on each plant was recorded.

Analysis of variance using type 1 error rate of 0.05 was used to analyze data and treatments means were partitioned by Duncan's new multiple range test.

Experimental Results: Ten cultivars of maturity group III were evaluated in the ongoing study. Cultivar 'Willomi' had the highest level of resistance, but was not significantly different from 'Wolverine', 'Fugi', and 'Kura'. Cultivar 'Williams' was most susceptible (Fig. 1).

Twelve cultivars of maturity group III were introduced this year. Those most resistant were PI 404,182, PI 88,310, and PI 54,615-1, while PI 438,496-3 was least resistant (Fig. 2).

In maturity group IV, 21 cultivars were studied in the ongoing study. The most resistant was 'SATO', but was not significantly different from 'Ware' and 'Funk Delicious'. 'Hahto/Mich' was most susceptible (Fig. 3 shows selected cultivars from least to most resistant.)

Fifteen cultivars of maturity group IV were introduced this year. Those most resistant were 'Douglas', PI 417,472-C, 'Sparks', and PI 84,751, while 'Franklin' was least resistant (Fig. 4).

In maturity group V, 13 cultivars were evaluated in the ongoing study and 19 new introductions. Those that showed high levels of resistance were PI 417,159 of the ongoing study, and PI 417,141 and PI 416,982 of the new introductions. Susceptibility was shown by PI 417,052 of the ongoing study and PI 417,193 of the new introductions (Figs. 5 and 6).

We hope that these cultivars will be a good source for the development of high yielding vegetable soybean cultivars resistant to H. zea.

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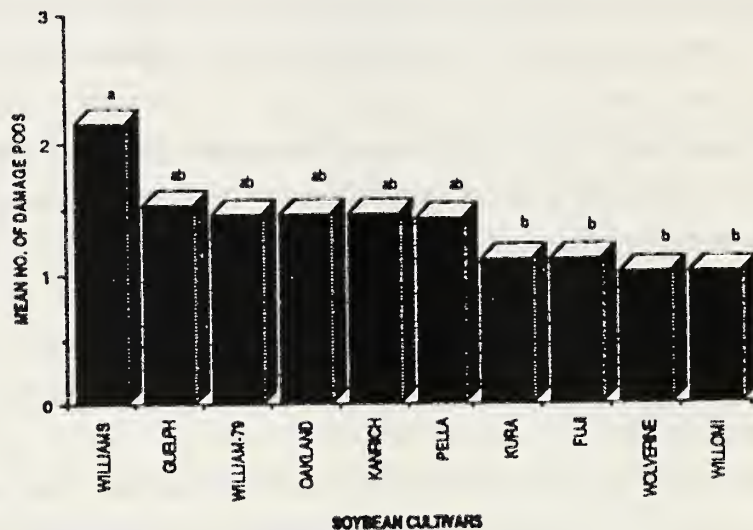


Fig. 1. Mean pod damage comparison for Maturity Group III.

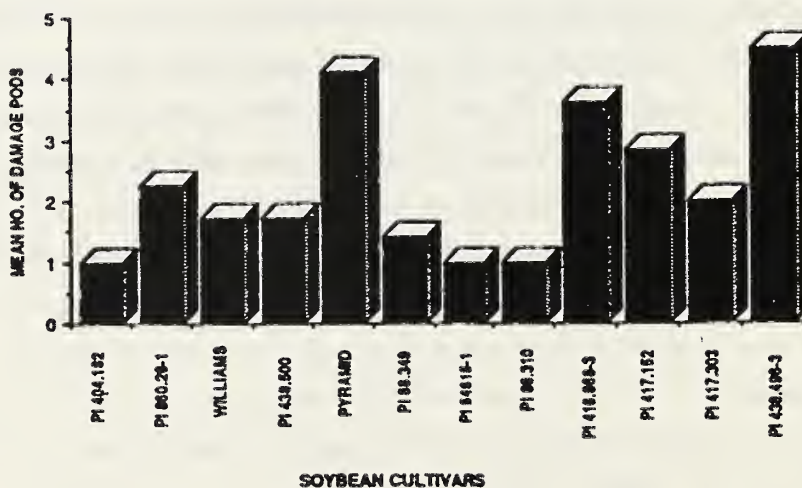


Fig. 2. Mean pod damage comparison for new introductions Maturity Group III.

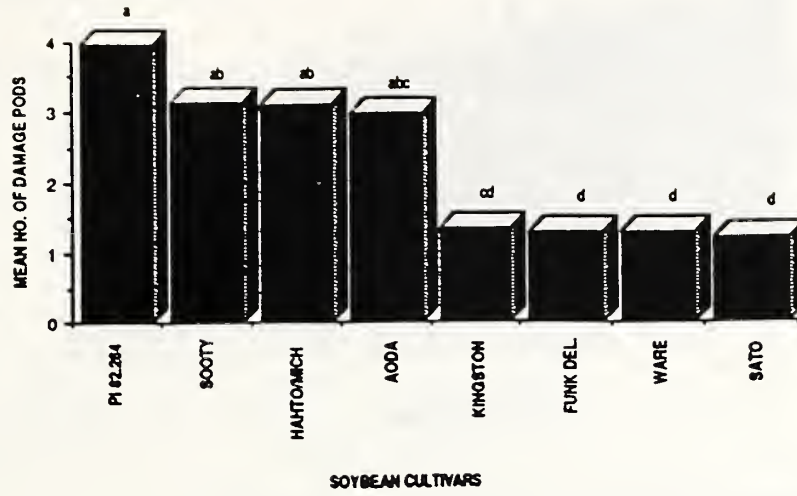


Fig. 3. Mean pod damage comparison for Maturity Group IV.

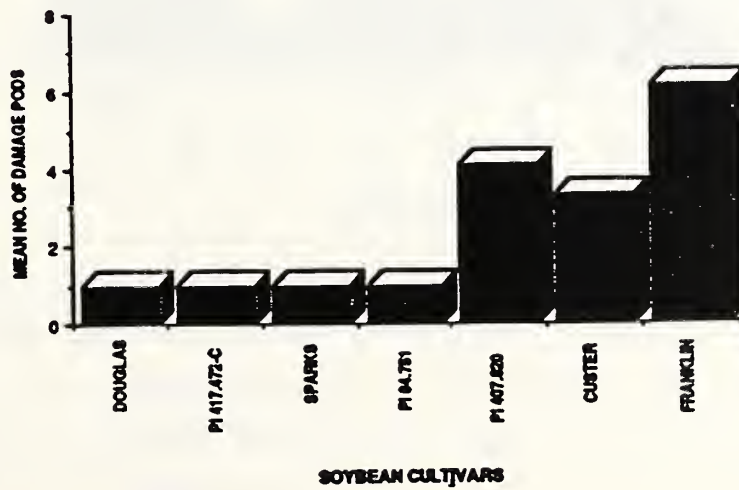


Fig. 4. Mean pod damage comparison for new introductions Maturity Group IV.

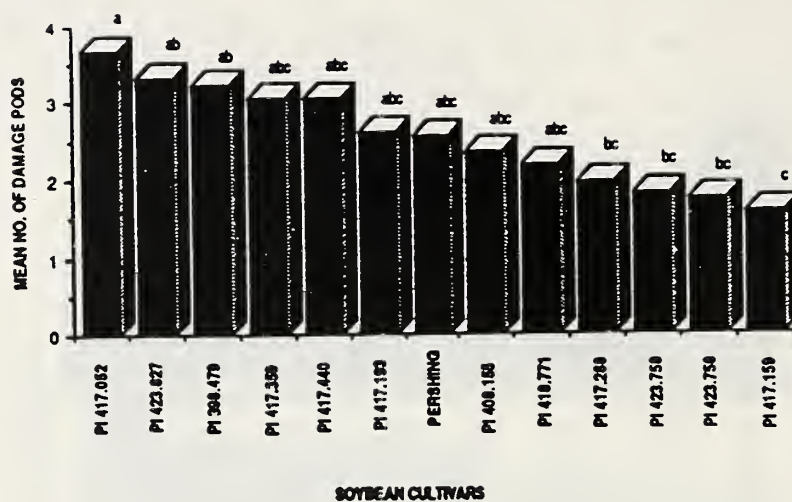


Fig. 5. Mean pod damage comparison for Maturity Group V.

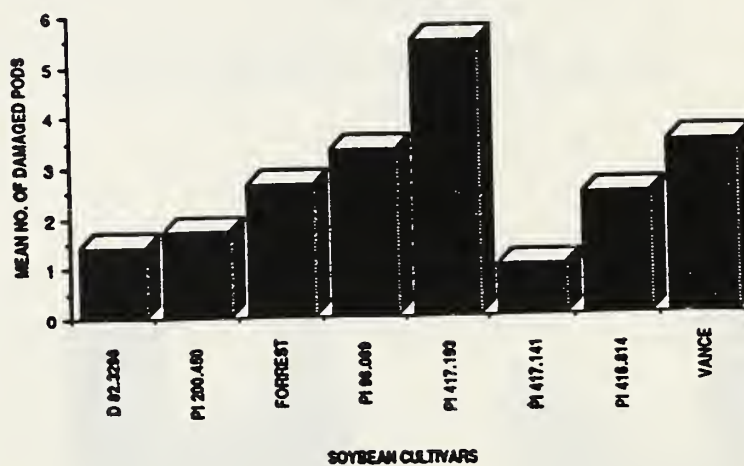


Fig. 6. Mean pod damage comparison for new introductions Maturity Group V.

H.W. Thompson

J.M. Joshi

R.B. Dadson

2) Leaf feeding resistance in soybean breeding lines to corn earworm
[*Heliothis zea* (Boddie)].

The increased importance of soybean damage by corn earworm and problems associated with insecticides have prompted researchers to develop insect resistant soybean cultivars. The discovery of soybean plant introductions PI 229,358 and PI 227,687 with resistance to damage by foliar feeding insects including corn earworm, in the 1970's (van Duyn et al., 1971; Clark et al., 1972) has generated interest in the development of resistant soybean cultivars. Plant breeders in the last three decades have attempted to increase the natural resistance of soybean cultivars to corn earworm and other insect pests by incorporating the resistance factor from the PIs. The transfer of resistance to improved breeding lines has been successful (Hatchett et al., 1979; Hartwig et al., 1984; Burton et al. 1986), but the progress has been slow in developing insect-resistant cultivars with acceptable agronomic qualities such as high yield levels and resistance to other major soybean pests. Therefore, a laboratory study was undertaken to evaluate the effect of antibiosis in soybean breeding lines on the larvae of corn earworm. The breeding lines were derived from crosses between PI 229,358 and PI 227,687 and commercial cultivars 'Essex' and 'Douglas'. The objective of this antibiosis study was to evaluate the level of incorporation of resistance into productive breeding lines.

Materials and Methods: Soybean seeds of each cultivar, PI, and breeding line (Table 1) were inoculated with *Rhizobium japonicum* and germinated in 20-cm diameter pots in the greenhouse. There were five plants in each pot per treatment, and at least 50 plants of each treatment were grown for each test. The bio-assays in all experiments were started when the plants were in the V5 or V6 development stages (Fehr and Caviness, 1977). The first fully expanded trifoliolate from the plant apex was excised for feeding tests. Each excised leaf was placed in a petri dish (10-cm in diameter), at the bottom of which a soaked filter paper was placed to keep the excised leaves fresh, and to avoid water loss from the larvae.

This research is a part of the Regional Research Project V, approved by the Association of Research Directors of the Historically Black Land Grant Colleges and Universities.

Table 1. Background of soybean breeding lines evaluated for antibiosis to corn earworm.

Breeding line	Parentage	Generation of Selection
D6872,006 ¹	Douglas x PI 227,687	F2
D6873,037	Douglas x PI 227,687	F3
D6873,022	Douglas x PI 227,687	F3
D6873,054	Douglas x PI 227,687	F3
D6873,019	Douglas x PI 227,687	F3
D6874,007	Douglas x PI 227,687	F4
D3583,010	Douglas x PI 229,358	F3
D3583,013	Douglas x PI 229,358	F3
D3584,009	Douglas x PI 229,358	F4
E6873,037 ²	Essex x PI 227,687	F3
E6873,042	Essex x PI 227,687	F3
E6873,010	Essex x PI 227,687	F3
E6872,003	Essex x PI 227,687	F2
E6874,006	Essex x PI 227,687	F4
E6872,001	Essex x PI 227,687	F2
E3581,002	Essex x PI 229,358	F2
E3583,019	Essex x PI 229,358	F3
E3583,022	Essex x PI 229,358	F3
E3582,013	Essex x PI 229,358	F2
E3582,001	Essex x PI 229,358	F2

¹D-indicates lines derived from crosses between Douglas and PI's.

²E-indicates lines derived from crosses between Essex and PI's.

Three newly hatched larvae were placed in each petri dish (containing a leaflet) with an artist's brush. Each experiment consisted of 24 replications for each treatment. The petri dishes were placed in a randomized complete block design and were kept at room temperature at $24\pm 2^{\circ}\text{C}$. The larvae were reduced to one on the third day and the leaves in the dish were replaced every 24 hours. The larvae were weighed on day 7, except in experiment 4 where the same was done on day 12. Recent studies have indicated that larval weights taken after 5 and 8 days of development gave an accurate and time-efficient measurement of soybean resistance (Beach and Todd, 1988) to soybean looper (*Pseudoplusia includens*) and velvetbean caterpillar (*Anticarsia gemmatilis*). Other parameters, such as weight gain and pupal weight, measured late in the development, are more time-consuming and are possibly less accurate indicators of antibiosis (Beach and Todd, 1988). Therefore, larval weight at an intermediate stage of larval development was considered a good indicator of soybean resistance to corn earworm during the current tests.

Table 2. Mean weights of corn earworm larvae in different tests in certain soybean cultivars and breeding lines.

Cultivar or line	Larval weights (mg)			
	Tests 1	Test 2	Test 3	Test 4
Douglas	7.4a ¹	9.6b	6.7b	150.0abcd
D6873,037	5.2bc	15.5a	-	115.4cde
D6873,022	4.2cd	6.6bc	2.7de	-
D6873,019	-- ²	-	-	163.9ab
D6872,006	-	-	-	124.4bcde
D6874,007	-	-	-	148.2abcd
D6873,054	-	-	-	53.7g
D3583,010	7.4a	16.2b	3.8cde	-
D3583,013	4.9cd	10.2b	5.9bc	-
D3584,009	-	-	-	178.8a
Essex	4.7cd	7.3bc	5.1bcd	170.4a
E6873,037	6.9ab	15.5b	9.6a	-
E6873,042	3.7cd	5.4bc	2.6de	-
E6873,010	-	-	-	156.9abc
E6872,001	-	-	-	114.1cde
E6872,003	-	-	-	97.4ef
E6874,006	-	-	-	116.5cde
E3581,002	-	-	-	114.1cde
E3582,013	-	-	-	125.9bcde
E3582,001	-	-	-	72.9fg
E3583,019	3.0de	7.7bc	2.4de	-
E3583,022	3.2cde	6.0bc	1.9e	-
PI 229,358	0.9f	4.0c	1.0e	-
PI 227,687	1.5ef	3.8c	1.3e	38.0g

¹Means followed by the same letter in a column are not significantly different (P<0.01).

² Indicated lines not included in various tests.

Experimental Results: Data indicate that antibiosis studies based on the larval weights of corn earworm in a series of tests generally showed significant differences between the PI's and commercial cultivars (Table 2; Tests 1, 3, and 4). Also, antibiosis studies showed significant differences between the breeding lines in all four tests. Mean larval weights derived from larvae feeding on Douglas lines indicated that D 6873,022 and D 6873,054 (Douglas x PI 227,687) were not significantly different from the resistant parent (Table 2; Tests 2, 3, and 4). Out of the progenies of Essex x PI's, the resistance of breeding line E 3583,022 was similar to that of the resistant

parent (Table 2). Lines D 3583,010 and E 6873,037 were the most susceptible lines (Table 2; Tests 1, 2, and 3).

Partial or intermediate resistance could play an important role in reducing the use of insecticides, resulting in reduced risks to the environment, increased growers' profits, and be of great benefit to pest management programs (Wiseman, 1985). This also indicates that the resistance in the PI's to corn earworm can be successfully incorporated into high yielding varieties. According to Kogan (1972), F1 plants of crosses between cultivar 'Bragg' and PI 229,358 exhibited an intermediate response to Mexican bean beetle. Although the genetic basis of resistance in the PI's is not fully understood (Hatchett et al., 1979), the resistance detected in our tests in breeding lines provide some evidence that the trait is transmissible.

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I. Javaid
J. M. Joshi
R. B. Dadson
M. Nobakht

3) Antibiosis studies on certain vegetable soybean cultivars to corn earworm
[*Heliothis zea* (Boddie)]

Soybean is being used extensively as a vegetable in some countries in the Asian continent. In the United States, fresh, frozen and canned forms of soybean products are also becoming popular. The soyfood industry is very much interested in vegetable soybean varieties. Due to the high demand for foods with no cholesterol, human consumption of soybean is likely to increase further. This nontraditional use of soybean can play an important role in supplying protein needs of the world population, including developing countries.

Insect resistance in soybean has become an important component in all breeding programs in the United States and a lot of effort has been directed toward the development of insect-resistant genotypes. But there is less information available concerning antibiosis of corn earworm in vegetable soybean genotypes. The objective of this study was to evaluate the relative degree of antibiosis in selected vegetable soybean genotypes.

Materials and Methods: Vegetable soybean genotypes evaluated for antibiosis to corn earworm were 'Fuji', 'Kim', 'Oakland', 'Peking', 'Sanga', and 'Toano'. 'Essex', a commercial cultivar, and two soybean plant introductions (PI 227,687 and PI 229,358) with known resistance to corn earworm, were used as resistant checks. Descriptions of the cultivars tested are given in Table 1. Seeds of each genotype were germinated in 20-cm pots and inoculated with Rhizobium japonicum. There were six plants per pot in each treatment and at least 50 plants of each genotype were grown for each test. Bioassay tests were started when the plants were in V5 and V6 (Fehr and Caviness, 1977) development stages. The first fully expanded trifoliolate from the plant apex was excised and each excised leaflet was placed in a petri dish (15-cm diameter). At the bottom of each petri dish, a soaked filter paper was placed to keep the leaves fresh. In each test, 24 replications of each treatment were included. The petri dishes were arranged in a randomized

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complete block design and were kept at room temperature ($26\pm 2^{\circ}\text{C}$). Three newly hatched larvae were placed in each petri dish with an artist brush. The larvae were reduced to one on the third day and the leaves in petri dishes were replaced daily.

Table 1. Source and maturity group of vegetable soybean cultivars evaluated for antibiosis to Heliothis zea.

<u>Cultivar</u>	<u>Source</u>	<u>Maturity Group</u>
Fugi	Japan	III
Kim	USA	III
Oakland	USA	III
Peking	China	IV
Sanga	China	IV
Essex	USA	V
Toano	USA	V
PI 227,687	Okinawa	VII
PI 229,358	Japan	VIII

In test 7, the larvae were weighed on day 12 and in all other tests the larval weights were recorded on day 7 only. According to Gary et al. (1985) resistance to Heliothis zea influences early development rates much more than the ultimate rates. They also indicated that pupal weights are not as greatly influenced by resistance as larval weights. Also, Beach and Todd (1988) pointed out that larval weights between 5 and 8 days of development are accurate and time-efficient measurements of soybean resistance to soybean looper (Pseudoplusia includens) and velvetbean caterpillar (Anticarsia gemmatilis). An intermediate stage of larval development (about 7 days old) was regarded as a good indicator of soybean resistance to corn earworm in our tests.

Results and Discussion: There were significant differences ($P < 0.01$) in the weights of larvae when fed upon different genotypes (Table 2). However, none of the genotypes tested showed more antibiosis to corn earworm than the resistant PI's. Mean weight of corn earworm larvae for all 7 tests for different cultivars indicated that larvae reared on cultivar Sanga gained maximum larval weight (Table 2). In earlier studies Sanga showed a high level of nonpreference to corn earworm in the field (Thompson et al., 1990). However, in the current test it showed least antibiosis. Also, the weights of

larvae fed upon cultivars Peking and Oakland were not significantly different from cultivar Sanga. Therefore, cultivars Sanga, Peking and Oakland appear to be comparatively more susceptible to corn earworm (Table 2). The antibiosis tests also showed that weights of the larvae fed on cultivars Fuji, Kim, Toano, and Essex were generally not significantly different from the weights of the larvae fed on the two PI's (Table 2). In earlier studies Essex was also reported to have intermediate levels of nonpreference and antibiosis to corn earworm (Joshi, 1980, 1981). There is an indication that these vegetable soybean cultivars exhibited a moderate level of resistance to corn earworm. Detection of moderate resistance in some vegetable soybean cultivars as indicated in our tests is important. In fact, complete resistance of a genotype to insect pests should not be the main goal of breeding programs (Kea et al., 1978). Moderate levels of resistance that permit the use of lower dosages of insecticides are economically and ecologically valuable. Indeed, low levels of resistance can be of great benefit to pest management programs. According to Kea et al. (1978), *Heliothis zea* feeding on resistant genotypes ED 73-371 were found to be more susceptible to insecticide methyl parathion and also to a microbial pathogen (*Bacillus thuringiensis*). This further illustrates the value of moderate levels of insect resistance. Vegetable soybean cultivars having some antibiosis resistance may have considerable

Table 2. Mean weights of corn earworm larvae (mg) in different tests on vegetable soybean cultivars.

Cultivar	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	X
Essex	13bc	6abc	11a	11bc	5c	72a	97bc	31abc
Fuji	33a	4bcd	6abc	32a	6bc	- ¹	68bc	24abc
Kim	22ab	7ab	3bc	23ab	5c	58ab	-	20abc
Oakland	31a	8a	9a	17b	12b	75a	127ab	40a
Peking	18ab	9ab	9ab	34a	20a	77a	117ab	40a
Sanga	19ab	7a	10a	18b	23a	82a	166a	47a
Toano	12bc	4abcd	2c	11bc	3c	-	119ab	25abc
PI 227,687	14bc	3cd	1c	12bc	8bc	39b	35c	16bc
PI 229,358	5c	2d	1c	6c	2c	8c	-	4c

Means followed by the same letter in a column are not significantly different ($P < 0.01$) according to Duncan's new multiple range test.

¹ Indicates missing data.

value in suppressing corn earworm populations and preventing economic damage.

The results of antibiosis tests also confirm the previous field evaluations in which cultivar Fuji was found to be comparatively more resistant (high nonpreference) to corn earworm within the vegetable soybean cultivars belonging to the same maturity group (Thompson et al., 1990). Additional evaluations of vegetable soybean cultivars that have shown moderate resistance to corn earworm are suggested for other key pests of soybeans. There is also a need for further antibiosis evaluations of more vegetable soybean cultivars with good agronomic characters.

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J. M. Joshi

I. Javaid

R. B. Dadson

M. Nobakht

UNIVERSITY OF TENNESSEE
Plant Molecular Genetics
Institute of Agriculture
and Center for Legume Research
Knoxville TN 37901-1071

1) High-resolution DNA Amplification Fingerprinting (DAF): Detection of amplification fragment length polymorphisms in soybean using very short arbitrary oligonucleotide primers.

The use of DNA "fingerprinting" has broad application in plant science. DNA fingerprints are normally produced using probes that hybridize to genomic DNA and detect alleles from single or multiple loci as restriction fragment length polymorphisms (RFLPs). These RFLPs are visualized as bands resembling the bar codes of supermarket products. Amplification of single-locus minisatellites by the polymerase chain reaction (PCR) using specific flanking primers (Jeffreys et al., 1988; Boerwinkle et al., 1989; Horn et al., 1989; Tautz, 1989, Jeffreys et al., 1990) and mapping of repeated sequences within these amplified regions (Jeffreys et al., 1990) has also been used to produce individual-specific fingerprints. In all these studies, detection of DNA polymorphisms required considerable experimental manipulation and prior knowledge of DNA sequence. Single primers of arbitrary nucleotide sequence have been used to amplify genomic DNA from a wide variety of organisms to reveal DNA polymorphisms (Williams et al., 1990; Welsh and McClelland, 1990; Caetano-Anolles et al., 1991). We found that DNA amplification with a single arbitrary primer as short as 5 nucleotides in length can produce detailed and relatively complex DNA profiles. This approach, DNA amplification fingerprinting (DAF), allows the easy detection of amplification fragment length polymorphisms (AFLPs) when amplification products are resolved by polyacrylamide gel electrophoresis and stained with silver (Caetano-Anolles et al., 1991). DAF has numerous advantages. It is simple and rapid yet sensitive, does not use cloned probes, and is independent of prior DNA sequence information. Here we describe the application of DAF to detect AFLPs in soybean.

Materials and Methods: Genomic DNA was prepared using established procedures (Dellaporta et al., 1983). Amplification was done in a 20-100 µl

reaction volume with 0.1-0.2 pg/ul⁻¹ of template DNA, 12 µM of primer, 200 µM of each dNTP (Pharmacia) and 0.025 unitsul⁻¹ of DNA polymerase from Thermus aquaticus (Ampli-Taq or Taq polymerase, Perkin-Elmer/Cetus), in reaction buffer [10 mM TrisHCl (pH 8.3), 50 mM KCl and 2.5 mM MgCl₂]. The reaction mix was overlaid with 1-2 drops of mineral oil, incubated for 5 min at 95°C, and amplified in an Ericomp thermocycler for 30-45 cycles (1 sec at 95°C, 10 sec at 30°C and 10 sec at 72°C, with a heating rate of 23°C/min⁻¹ and a cooling rate of 14°Cmin⁻¹). DNA amplification products were analyzed by electrophoresis in polyester-backed polyacrylamide gels and silver stained as described (Bassam et al., 1991).

Results: Although a mixture of several primers can be used, only one short oligonucleotide typically 8-10 bases in length, is required. Temperature cycling in the presence of a thermostable DNA polymerase produced a range of short amplified DNA products of different length. The spectrum of products was characteristic, but changed with each primer and template combination. When separated using polyacrylamide gel electrophoresis, the amplified products resolve into a banding pattern or "fingerprint". Their number ranged from less than 10 to over a hundred (Caetano-Anolles et al., 1991). Using this approach, we have obtained highly reproducible fingerprints from genomic DNA of a wide variety of organisms ranging from viruses to humans. It should be noted that agarose gel electrophoresis and ethidium bromide staining of amplification products detects only the few major fragments (Williams et al., 1990; Welsh and McClelland, 1990). Thus, considerable loss of information can occur if suitable fragment separation and detection procedures are not employed.

DAF can be tailored to produce patterns of varying complexity by changing the primer sequence. To illustrate this a set of 20 arbitrary 10-base oligonucleotide primers with similar G+C content was used to amplify genomic DNA from soybean cv. 'Bragg'. The resulting fingerprints were compared to those generated with the same primers from a bacterium (Staphylococcus aureus) and a caucasian human (Fig. 1). The number of amplification products was quite variable, ranging from 0 to 19 for bacterial DNA, from 2 to 49 for soybean DNA, and from 0 to 60 for human DNA, but correlated with genome size. Fingerprint tailoring can be of great value. Relatively simple patterns are suitable for genetic mapping, while more complex patterns with higher information content are most useful for genotyping.

DNA polymorphisms were detected within the plant genus Glycine subgenus

Soja between the cultivated soybean and the wild annual species of G. soja (Sieb. and Zucc.). More importantly, we have detected polymorphisms between soybean cultivars. For example, as many as 6 AFLPs were detected between cultivars Bragg and 'Peking' using the primer sequence CGCGGCCA. Because soybean is a self-pollinator with a narrow genetic base (most cultivars share a common ancestry and individuals are highly homozygous), it should be noted that DNA polymorphisms using established fingerprinting techniques are difficult to detect (Doyle and Beachy, 1985; Doyle, 1988; Apuya et al., 1988; Keim et al., 1989).

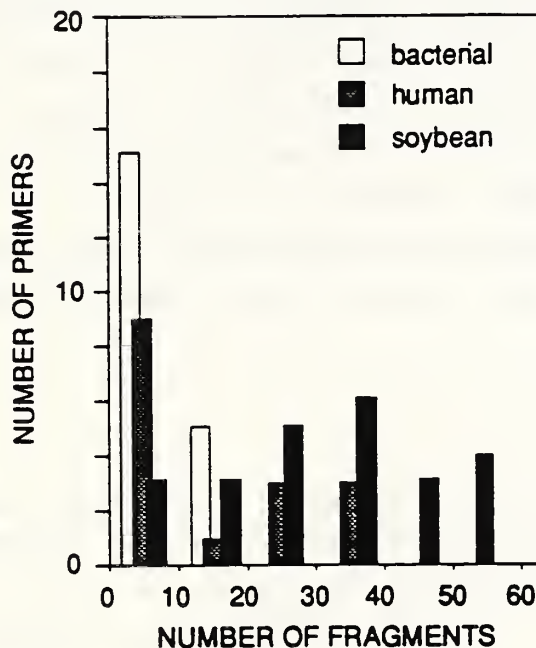


Figure 1. Variation in the number of amplified products obtained with different primers. Genomic DNA from S. aureus, soybean cv. Bragg and a caucasian human was amplified with each of 20 different 10-base oligonucleotides with G+C contents between 60-70%.

Discussion: Conventional DNA fingerprinting detects genetic differences as base pair changes that alter restriction endonuclease sites within defined loci (Botstein et al., 1980). DAF uses a single primer to amplify portions of a DNA template extending from a priming site sequence to its specular complementary sequence. Since multiple unspecified priming sites on each DNA

strand generate a range of extension products, our procedure detects polymorphisms that arise from changes in DNA sequence at arbitrary primer-defined sites in the genome. These changes can be single-base permutations, the deletion/insertion or inversion of priming sites, or the deletion/insertion of segments between priming sites. Such changes manifest in the number and length of amplified products. A general observation is that bands fall into two categories; those that are phylogenetically conserved and those that are individual-specific. Thus we presume primer sites are randomly distributed along the target genome and flank both conserved and highly variable regions.

AFLPs can function as genetic markers constituting sequence-tagged sites (Williams et al., 1990). Since prior knowledge of DNA sequence is not required, a set of universal and very short primers can be used to generate high marker density maps of eukaryotic and prokaryotic genomes. High-resolution DAF will also be useful for the identification of near-isogenic soybean lines and cultivars, and in phylogenetic studies.

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Gustavo Caetano-Anolles

Brant J. Bassam

Peter M. Gresshoff

2) Leaf protein analysis of Glycine max (L.) Merr. cv. Bragg and its supernodulating mutant nts382.

The research emphasis in our laboratory is to elucidate the mechanism of autoregulation of nodulation that occurs in soybean when infected with Bradyrhizobium. Autoregulation of nodulation is manifested by the systemic suppression of further symbiotic cell divisions in ontogenically younger root tissue in the form of arrested infections (Caetano-Anolles and Gresshoff, 1991). Major regulation of nodulation occurs during nodule primordium development (Caetano-Anolles and Gresshoff, 1990; Gerahty et al., 1990) and involves active signal molecules (shoot-derived inhibitor) that as yet have not been identified. Grafting studies by Delves et al. (1986) showed that all supernodulating mutants of soybean controlled their nodule phenotype through the action of the shoot. Split roots showed that prior inoculation of one side suppressed nodulation on the other side in the wild type, but not in nts plants (Olsson et al., 1989). More recently, Delves et al. (1991) showed that the leaf and not the shoot apex is responsible for the autoregulation response. These lines of evidence led us to investigate polypeptide changes in leaves of cv. 'Bragg' concomitant with infection by Bradyrhizobium japonicum USDA110. Using two dimensional (2-D) gel analysis we investigated in vitro translated proteins and total in vivo proteins of wild-type Bragg and its supernodulating mutant nts382 (which has diminished autoregulation of nodulation) at various time points after inoculation with strain USDA110 (Sayavedra-Soto et al., 1991).

Materials and Methods: Leaf tissue was obtained from Glycine max cv. Bragg and its mutant nts382. Plants were grown in plastic pouches, or in pots with vermiculite or a 1:1 mixture of sand/vermiculite. Pouches were watered with Jensen's nutrient solution, while pots were watered with a modified Hoagland nutrient solution supplemented with 0.5 mM nitrate, added to permit healthy development of uninoculated plants (Olssen et al., 1989) yet not sufficiently high to inhibit nodulation. Plants were inoculated with Bradyrhizobium japonicum strain USDA110 (0.5 ml per plant at 10^8 cells/ml or 50 ml of the same culture was diluted to 4 liters with nutrient solution and added to the pot-grown plants). Leaf tissue was extracted immediately for

total RNA or kept at -70°C until used.

Total RNA Isolation: Total RNA was obtained by the guanidinium method as described (Davis et al., 1986) with minor modifications. At least three similar pouch-grown plants or at least 10 pot-grown plants were used for each RNA extraction. The sampled tissue included trifoliolates, primary leaves, petioles and apices. Total RNA was extracted from tissue at days 0, 2, 6, and 25 days after inoculation and was translated in vitro using wheat germ extract or a rabbit reticulocyte lysate (Promega) and an amino acid mixture containing [³⁵S]methionine.

2-D gel Electrophoresis: Protein products were analyzed by 2-D gel electrophoresis (Hochstrasser et al., 1988). Approximately $5 - 9 \times 10^5$ cpm were loaded per gel as determined from TCA-precipitated samples. Electrophoresis was performed in a Mini-protean II 2-D cell apparatus (Bio-Rad). The dried gels were exposed to X-OMAT AR film (Kodak) at -70°C for 7 to 15 days. Total in vivo proteins were extracted as described (Flensgrud and Kobro, 1989).

Results and Discussion: The growth conditions were standardized to account for any differences observed in the polypeptide changes due to inoculation. Since cultivar Bragg has a high Mn toxicity the vermiculite was mixed with autoclaved sand. We translated RNA populations in vitro and isolated total proteins to monitor the polypeptide patterns in leaves of G. max upon inoculation with B. japonicum. Protein patterns of total RNA and polyA+RNA of the same harvested sample were identical. Great care was taken to base conclusions on repeated gels and replicated extractions. Variations caused by technical conditions were kept to a minimum.

The regulation of nodulation is elicited during early cortical cell divisions occurring within 12 to 48 hours after exposure to rhizobia (Caetano-Anolles et al., 1991). On comparing autoradiographs of in vitro translated proteins from wild-type and nts382 plants two days after inoculation, no differences due to inoculation or mutation were detected. Some developmental changes were seen. Several polypeptides were found to be invariant and were used as reference proteins within gels to allow comparisons between samples. Two polypeptides (D1 and D2) decreased by eight days, showed a transient peak of abundance at 10 days after inoculation and then decreased gradually again. Another polypeptide (D3) increased in abundance by 10 days. Nodule formation is profuse 10 days after inoculation resulting in an increased demand for

nitrogen translocation to the root (Hansen et al., 1990). Some of this nitrogen may stem from the degradation of leaf proteins. Comparing samples of wild-type and nts382 plants 25 days after inoculation showed that although overall polypeptide patterns did not change, several polypeptides (I1, I2, I3

Table 1. Reference (R), developmental (D) and inoculation (I)-related polypeptides of soybean leaves.

Polypeptide	Approximate molecular weight (kD)	Apparent isoelectric pH ¹
D1	40	6.6
D2	40	6.7
D3	35	6.4
I1	42	5.7
I2	26	6.4
I3	27	6.6
I4	15	6.2
I5	15.5	6.4
R1	44	5.8
R2	42	5.9
R3	21	5.6
R4	16	5.7

¹Assuming linearity of pH gradient. Calculated by intrapolation, not direct measurement.

I4 and I5) consistently changed in the levels of expression (intensity). Polypeptide I1, which showed constant abundance for the first 10 days, decreased in intensity in uninoculated Bragg relative to inoculated wild type, but showed the reverse in nts382. Polypeptide I2 appeared to increase in relative intensity in inoculated plants compared with uninoculated plants in both wild type and mutant. Neither I1 nor I3 changed at earlier time points. Polypeptides I4 and I5 increased in inoculated plants at 25 days but did not do so during the first 16 days in both nts382 and Bragg plants. Table 1 shows the approximate molecular weights and presumed pH at their isoelectric points of the discussed polypeptides.

These changes in polypeptide patterns may be correlated to nodulation and subsequent nitrogen fixation. Concomitant polypeptide changes are expected from leaf tissue because translocation of large amounts of ureides, allantoin and allantoic acid are translocated from nitrogen-fixing roots. The analysis

of several replicate translations from different plant sets allowed us to discern clearly between nodulation/developmental changes. Comparing the 2-D polypeptide pattern of total leaf protein, 4 days after inoculation, showed that there were no differences due to inoculation or mutation in the two patterns, further confirming the results from the in vitro translated proteins.

The lack of protein differences due to inoculation at time points earlier than eight days can be interpreted in several ways: (a) the infection signal from roots is modified by a constitutive system in the leaves; (b) the tissue responsible for the regulation of nodulation is only a minor proportion of the total biological sample and thus quantitatively different mRNA populations or their products are underrepresented in the analysis; (c) the polypeptide changes involved in the regulation or the formation of the nodule are well below the detection limits of gel electrophoresis; (d) the polypeptide altered in the supernodulation mutant (i.e., the nts gene product) is still present but lacks biochemical or regulatory activity or the nts mutation is not absolute and the difference in the presence of the gene product is too subtle; (e) the nts mutation as well as the onset of autoregulation are not associated with major pleiotropic changes in the leaf biochemistry and physiology.

Conclusions: Polypeptides associated with development either showed transient peaks of expression or gradually increased/declined over the 25-day experimental period. These quantitative changes may represent differential mobilization of structural and metabolic resources during leaf growth and development. Changes in levels of expression of some polypeptides were also associated with inoculation but these occurred 25 days after inoculation, when the plant was well nodulated and fixing nitrogen. Inoculation with near isogenic Fix⁺ Bradyrhizobium could resolve the question whether these changes in levels are due to nodulation or nitrogen fixation per se.

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L. A. Sayavedra-Soto

S. A. Angermuller

R. Prabhu

P. M. Gresshoff

UNIVERSITY OF GEORGIA
 Department of Agronomy
 Athens GA 30602

1) Selection for improved regeneration capacity in soybean.

A prerequisite for improvement of soybean by genetic engineering technology is an efficient transformation and regeneration system. In the case of soybean, one important aspect of such a system is the frequency of regeneration from tissue culture via the formation of somatic embryos. Genetic control of in vitro responses such as regeneration has been demonstrated in many species, including legumes such as alfalfa (Chen and Marowitch, 1987) and white clover (Mohapatra and Gresshoff, 1982; Bhojwani et al., 1984). One study indicated that regeneration capacity in soybean is also under genetic control, since a genotype with high embryogenic capacity, 'Manchu', transferred some regeneration capacity to F1 progeny (Parrott et al., 1989). All soybean genotypes tested have regenerated via somatic embryogenesis, but frequency of response among genotypes has varied as much as 100 fold (Komatsuda and Ohyama, 1988; Parrott et al., 1989).

Northern US cultivars (Maturity Groups 00 to IV) are derived from a small number of ancestral genotypes (Delannay et al., 1983). An examination of the regeneration capacity (RC) of these ancestral genotypes revealed two superior regenerators, Manchu (PI 30,593) and 'A.K. Harrow'. A subsequent pedigree analysis of recent cultivars revealed that the best regenerators from tissue culture had Manchu and/or A.K. Harrow in their background. The best cultivar of recent agronomic importance was 'Century' (Parrott et al., 1989).

Another report recently identified Japanese genotypes with a relatively high regeneration potential (Komatsuda and Ohyama, 1988). One genotype, 'Masshokutou (kou 502)', had embryogenic capacity similar to that of Manchu. The viny growth habit and small, brown seed of this genotype were described as characteristics of Glycine gracilis. Once considered a separate species, G. gracilis is now classified as a weedy form of G. max. This genotype is a potential source of regeneration capacity distinct from that of regenerable northern U.S. germplasm, and, therefore, selection for regeneration capacity higher than either of these sources may be possible. The objective of this study was to evaluate the effectiveness of selection for increased

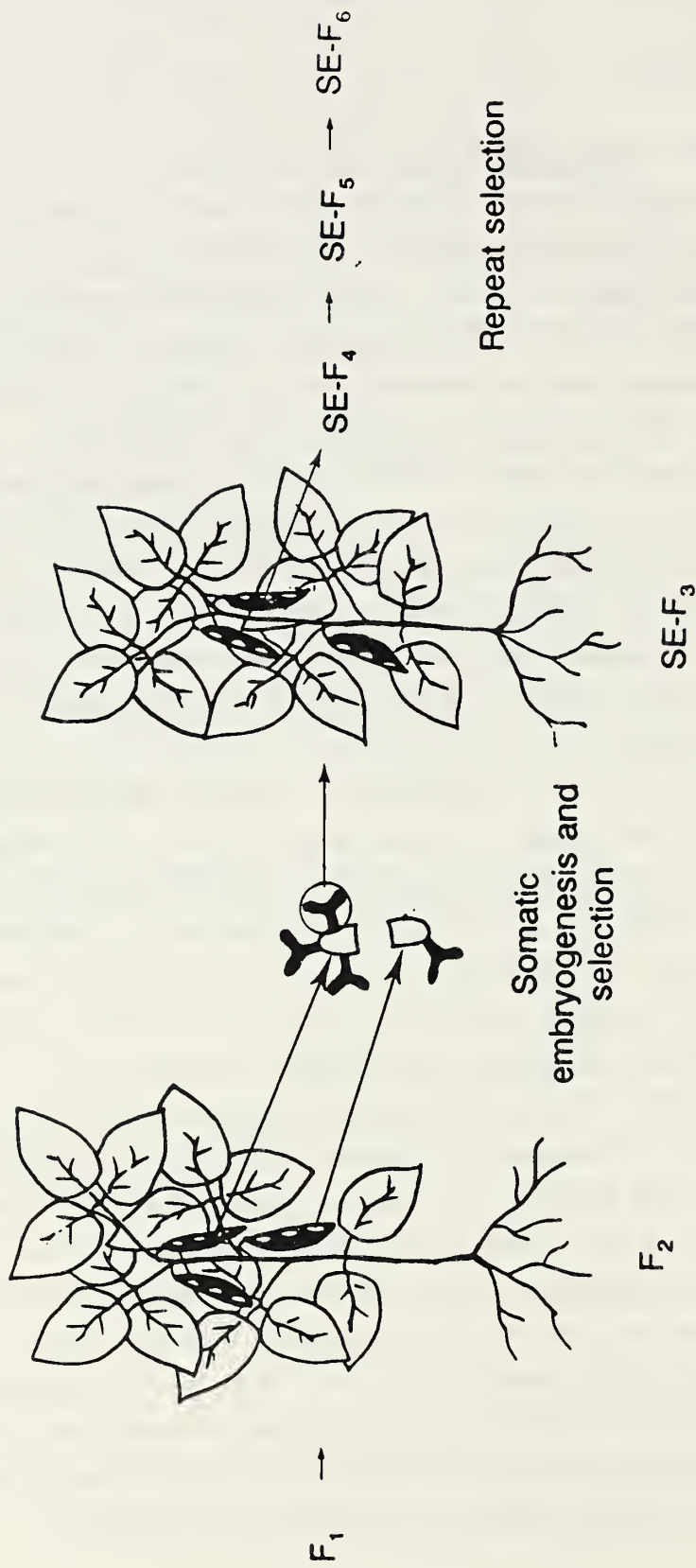


Figure 1. Terminology and selection scheme

regeneration capacity in a cross of parents representing diverse sources of regeneration capacity.

Materials and Methods: 'Century' was crossed with PI 417,138 [Masshokutou (kou 502)], and progeny of the F_2 SE- F_3 , and SE- F_4 generations were evaluated for production of somatic embryos/cotyledon (SE/COT) by an established protocol (Parrott et al., 1988). The "SE-F" terminology is used to indicate that given individuals or generations were derived from somatic embryos. Thus, an SE- F_3 plant represents an individual derived from a somatic embryo regenerated from an F_3 cotyledon. As no meiosis is involved, an SE- F_3 should have the same genotype as the cotyledon that produced it (barring mutations that may occur in culture). This terminology and the selection scheme are outlined in Figure 1. Briefly, 89 F_3 families were evaluated for somatic embryogenesis (SE/COT), and somatic embryos from the best 20 families were selected for conversion into plants. Fifteen SE- F_3 individuals representing nine F_3 families were successfully converted into plants. All 15 F_4 families were evaluated for SE-COT, and individual SE- F_4 embryos were selected for conversion based on two criteria: 1) they were from the best three F_4 families, and 2) they were from the best F_4 individuals (cotyledons) within the best three F_4 families. Seventeen SE- F_4 individuals selected by these criteria were converted and planted in the greenhouse. All 17 F_5 families were evaluated for SE-COT and individual SE- F_5 embryos were selected for conversion by the same criteria as in the SE- F_4 generation. For all generations, the number of cotyledons evaluated per family ranged from 2 to 254. Individual selections were made only from those families in which at least 30 cotyledons were evaluated.

Results and Discussion: The SE/COT was increased by selection (Table 1). The data suggest that transgressive segregation of genes conditioning regeneration capacity has occurred, since the regeneration capacity of the best F_3 family of Century x PI 417,138 was significantly higher than the regeneration capacity of the parents. This family has continued to outperform its parents through to the F_5 generation. We have provided parent data separately for each generation since absolute embryogenic frequencies typically vary among experiments. However, relative rankings of embryogenic frequencies among genotypes are usually constant with this protocol. No parents were evaluated concurrently with the F_4 generation, since we were unable to synchronize parents and SE- F_3 individuals with regards to greenhouse

acclimatization, maturity, and seed production.

The superior regenerants from this population will be further tested by concurrently evaluating the SE/COT of SE-F_{3,6} lines, parents, and the original F₂ population. The lines with high regeneration capacity should be useful for developing efficient transformation systems in soybeans.

Table 1. Average production of somatic embryos per cotyledon \pm standard error for three generations of Century x PI 417,138 and parents. ND = no data collected.

Parents		Generation	No. of families	Mean of all families	Mean of best family
Century	PI 417,138				
0.14 \pm 0.02	0.20 \pm 0.03	F ₃	89	0.14 \pm 0.01	0.55 \pm 0.16
ND	ND	F ₄	15	0.24 \pm 0.02	0.56 \pm 0.18
0.13 \pm 0.03	0.82 \pm 0.05	F ₅	17	0.97 \pm 0.04	2.11 \pm 0.18

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M. A. Bailey

W. A. Parrott

H. R. Boerma

VIRGINIA POLYTECHNIC INSTITUTE

and STATE UNIVERSITY

Crop and Soil Environmental Sciences Department

Blacksburg VA 24061

1) Inheritance of delayed flowering in soybeans.

Several reports of the inheritance of delayed flowering are available. Kiihl (1976) reported that delayed flowering under short-day conditions in 'Santa Maria' is controlled by recessive genes, but he did not indicate the number of genes involved. Hartwig and Kiihl (1979), using Santa Maria and PI 159,925 as the sources of delayed flowering, concluded that the trait was controlled by as many as three recessive genes. Azlon (1981), using PI 159,925 as the source, first suggested that delayed flowering under short-day conditions was controlled by a single recessive gene. However, he could not exclude the possibility of a second major gene since flowering date classes in the populations he studied often were not discrete. That the delayed flowering trait is controlled by a single recessive gene was also supported by the data obtained by Malo (1986) who found that most F₂ progenies from a cross of early x late flowering produced a 3 early : 1 late ratio, using PI 159,925 as the delayed flowering source. However, three of the 26 progenies appeared to segregate for more than one major gene. Hinson (1989), describing delayed flowering under short-day conditions as 'long juvenility' and using materials derived from PI 159,925, concluded that the trait was controlled by one recessive gene. However, he also indicated that the inheritance of delayed flowering may be more complex, due to the presence of more than two classes of flowering and maturity dates in a few late generation segregating rows. Bidja Mankono (1988) reported that delayed flowering derived from PI 159,925 was controlled by a single recessive gene, but found evidence also for a dominant gene of unknown source that delays flowering. He studied his segregating F₂ and advanced populations at three planting dates in June, July, and August in the field in Florida. Therefore, other known genes which regulate maturity date and are strongly influenced by photoperiod could have influenced his results.

While there appears to be fairly good agreement that long juvenility is controlled by a single recessive gene, there are enough exceptions to make

additional inheritance studies worthwhile. There also is some concern that other maturity genes might be confounding the classification of plants for juvenility type, especially in field studies. The objective of this study was to determine the inheritance of the delayed flowering trait in soybeans under controlled short-day conditions.

Materials and Methods: The parents used in the study included 'Essex', a Maturity Group V cultivar, and two pairs of isolines obtained from Dr. K. Hinson at the University of Florida, Gainesville. F85-1226 and F85-8417 are the delayed flowering isolines of F85-1221 and F85-8416, respectively. F85-1221 and F85-8416 are similar to Essex in maturity in the field. Both pairs of isolines were selected for delayed flowering in the field in the F8 generation of the cross [Kirby x (Forrest (3) x D77-12480)] x Will. D77-12480 is from the cross Tracy x (Hill x PI 159,925). PI 159,925, a plant introduction from Peru, is the original source of the delayed flowering trait.

Each of the Florida lines was crossed with Essex, which served as the pollen parent so flower color could be used as an F1 marker. Crosses were also attempted between the members of both pairs of isolines, but seeds were obtained only on the F85-1226 x F85-1221 cross. All crosses were made in the field at Blacksburg VA in 1989.

One to three F1 plants per cross, depending on available seed, were grown under extended photoperiod in the greenhouse at Blacksburg during winter and spring, 1990. Plants resulting from self-pollination were distinguished from F1's by hypocotyl color in appropriate crosses and verified at flowering time. Essex has purple flowers and hypocotyls and each of the isolines has white flowers and green hypocotyls. Genetic markers were not available to distinguish hybrids from self-pollinations for the F85-1226 x F85-1221 cross, so F2 segregation for flowering behavior served as a marker.

All the F2 populations were grown in the greenhouse during summer, 1990. Six seeds were planted per pot and thinned to four seedlings seven days after emergence. Eight to 12 plants of each parent were grown to serve as checks for classifying F2 plants. Half the pots from each F1 family and parental check were grown under continuous short-days (treatment 1) and half were treated similarly for eight days and then moved to 16 h long days (treatment 2). Both treatments were intended to distinguish delayed flowering plants from normal flowering plants. In treatment 1, the delayed flowering plants were expected to flower about four to five days later than normal ones (the difference

between long juvenile and normal juvenile periods determined in previous experiments). Treatment 2, also based on previous experiments, was designed to induce flowering in normal-juvenile plants, but not induce long juveniles, thus greatly expanding the difference in flowering dates of the two types.

For the short-day treatment, plants were covered with light-excluding black cloths at 7 p.m. and uncovered at 9:30 a.m. Long days were created by extending the natural daylength to 16 h with artificial lighting. The maximum daytime temperatures in the greenhouse were about 32°C and night temperatures were about 5-7°C lower.

Dates of planting and emergence were recorded. Plants were inspected every day and flowering dates were recorded. Days from emergence to first flowering was calculated for each plant. Chi-square analyses were performed on F2 data to test goodness-of-fit to expected segregation ratios. F1 families of the same cross were tested for homogeneity.

Results and Discussion: The short-long day treatment did not work as expected because the high temperatures compressed the juvenile period of all plants to less than eight days. Thus, most plants had already been induced to flower at eight days after emergence, but differences in days to flower between long-juvenile and normal-juvenile plants were still evident. The long-juvenile and normal parents were clearly distinguishable under both treatments, with no class overlap, and almost all the F2 plants were within the ranges of the parents. Observed segregation of F2 plants was very similar under both treatments, so the data were combined.

The F2 plants from both families of F85-8416 x Essex and the single family of F85-1221 x Essex all bloomed early (Table 1), as expected, since all three parents are similar in length of the juvenile phases. These results suggest that F85-8416, F85-1221 and Essex contain the same allele controlling the length of the juvenile period and they are homozygous.

Table 2 shows the segregation obtained from the F2 of the long-juvenile x normal-juvenile crosses. Each family, except one, provided a good fit to a 3 early : 1 late ratio. The p value for the one exceptional family was 0.05-0.02, but since the pooled data from F85-1226 x Essex was a good fit to a 3:1 ratio and the three families were not significantly different, it was assumed that each family was exhibiting single-gene segregation. These results suggest that delayed flowering is controlled by one locus with two alleles with long juvenility being recessive. This is in basic agreement with Malo

(1986), Bidja Mankono (1988), and Hinson (1989), who also reported that long juvenility is controlled by one recessive gene. The controlled short days provided very good discrimination between normal and long-juvenile plants, with no apparent confusion caused by segregation of genes controlling maturity.

Table 1. Flowering response of F2 plants from crosses between normal-juvenile isolines and Essex, when grown under short days in the greenhouse.

Cross	Family	Number of plants	
		Early	Late
F85-8416 x Essex	1	28	0
	2	25	0
F85-1221 x Essex	1	38	0

Table 2. Flowering response of F2 plants from crosses between long-juvenile and normal-juvenile parents when grown under short days in the greenhouse.

Cross	Family	Number of plants		$X^2(3:1)$	P
		Early	Late		
F85-8417 x Essex	1	24	5	0.931	0.50-0.30
	2	20	9	0.563	0.50-0.30
	3	<u>17</u>	<u>6</u>	<u>0.014</u>	0.95-0.90
Total				1.508	
Pooled (3:1)		61	20	<u>0.004</u>	0.95-0.90
Homogeneity (2 df)				1.504	0.50-0.30
F85-1226 x Essex	1	31	11	0.032	0.90-0.80
	2	29	11	0.133	0.80-0.70
	3	<u>24</u>	<u>16</u>	<u>4.800</u>	0.05-0.02
Total				4.965	
Pooled (3:1)		84	38	<u>2.459</u>	0.20-0.10
Homogeneity (2 df)				2.506	0.30-0.20
F85-1226 x F85-1221	1	26	7	0.253	0.70-0.50
	2	<u>23</u>	<u>8</u>	<u>0.011</u>	0.95-0.90
Total				0.264	
Pooled (3:1)		49	15	0.083	0.80-0.70
Homogeneity (1 df)				0.181	0.70-0.50

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D. Sumith de Z. Abeysiriwardena

Glenn R. Buss

UNIVERSITY OF COLORADO

Department of Molecular, Cellular and Developmental Biology

Campus Box 347

Boulder CO 80309

1) Behavior of the *ms2* mutation in an interspecies cross between *G. max* and *G. soja*.

The male-sterile, female-fertile mutation, *ms2*, of soybean causes degeneration of tetrads as well as aberrant development of the tapetum and anther wall layer (Graybosch et al., 1984; Graybosch and Palmer, 1985) resulting in shrunken, distorted anthers. Because the gene product of the *ms2* locus has not been identified, the molecular basis of sterility induced by this mutation is unknown. Moreover, the *ms2* locus has not yet been assigned to a genetic linkage group (Palmer and Kiang, 1990).

As a first step toward understanding the molecular basis of the *ms2* mutation, we have initiated a project to identify a marker DNA sequence tightly linked to the *ms2* locus through RFLP linkage analysis (Keim et al., 1990). Such a marker, which could be assayed on seedling plants before flowering, would also enhance the efficiency of a recurrent introgression breeding technique based on the *ms2* mutation (Specht and Graef, pers. comm.)

To assign the *ms2* mutation to an RFLP linkage group, we need an F₂ population that segregates sterile (*ms2 ms2*) plants and that is amenable to RFLP analysis. One parent required for the cross is a *G. max* plant (*ms2 ms2*) that carries the mutation. To ensure a sufficient level of DNA polymorphism for RFLP mapping, we chose *G. soja* (PI 468,916) as the second parent. This line is genetically diverse from *G. max* (Keim et al., 1989) and lacks a translocation common among *G. soja* accessions (Palmer et al., 1987); moreover, because this line was one of the parents for the F₂ population used by R. Shoemaker and his colleagues to generate the soybean RFLP map (Keim et al., 1990) most of the probes available to us from his lab will be useful. However, if the *ms2* mutation were not expressed as expected in our interspecific cross (i.e., if F₁ hybrids are not fertile or if the F₂ population does not segregate steriles) our F₂ population could not be used for RFLP mapping. We report here preliminary results indicating that the *ms2* mutation is expressed as expected.

Method: Stock seeds were obtained from the USDA Germplasm Collection, and plants were greenhouse-grown with a photoperiod of 14 h of light per day. Crosses were made between male-sterile G. max L7-401 [(ms2 ms2 wl wl); a nearly isogenic line (Williams⁶ x T259H)] and G. soja PI 468,916 (Ms2 Ms2 W1 W1). Two of the resulting F1 seeds were planted and about 900 F2 seeds were harvested from each F1 plant. For this study, we grew 82 plants from seeds of one F1 plant.

To determine expression of anthocyanin in W1 __ plants, we rated both hypocotyl color (purple or green) for all plants and flower color (purple or white) for plants that have flowered to date. Fertility/sterility ratings on plants that have flowered were based primarily on microscopic examination of anthers stained with acetocarmine; in some cases, a rating of fertile was confirmed by the presence of numerous normal pods.

Table 1. Segregation in the F2 population of the wl wl x W1 W1 cross in soybean.

Phenotype (genotype)	Observed	Expected
Purple (<u>W1</u> __)	62	61.5
White (<u>wl wl</u>)	20	20.5
Total	82	82
$X^2 = 0.016$ $df = 1$ $p = >0.90$		

Results: As a positive control of segregation in our F2 population, we were able to rate expression of anthocyanin expression because the G. max parent is homozygous for the wl mutation, and the G. soja parent is homozygous for the W1 allele. In the population of 82 plants, we observed 62 purple (W1 __) and 20 white (wl wl) phenotypes, which corresponds to a X^2 (3:1) of 0.016 and a probability of >0.90 (Table 1).

Because some of the F2 plants have yet to flower, we have data for segregation of sterility for only 69 plants. Of these, 52 were rated fertile, and 17 were rated sterile. X^2 (3:1) was 0.005, and probability was >0.90 .

Table 2. Segregation in the F₂ population of the ms2 ms2 x Ms2 Ms2 cross in soybean.

Phenotype (genotype)	Observed	Expected
Fertile (<u>Ms2</u> ____)	52	51.75
Sterile (<u>ms2 ms2</u>)	17	17.25
Total	69	69
$\chi^2 = 0.005$ $df = 1$ $p = >0.090$		

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Kathleen J. Danna

Richard N. Miranda

SOUTHERN ILLINOIS UNIVERSITY

Plant and Soil Science

Carbondale IL 62901

1) Inheritance of soybean SDS response in segregating F₅- and F₆-derived lines.

Introduction: Sudden Death Syndrome (SDS) causes a wide range of response in soybean cultivars. As of this writing, no immune genotypes have been documented. Cultivars and lines with soybean cyst nematode (SCN, Heterodera glycines Ichinohe) resistance have been shown to be more tolerant to SDS than SCN-susceptible lines. Lawrence et al. (1988), and Rupe (1988) described a connection of SDS with SCN. Roy et al. (1989) showed increased disease severity with the Fusarian solani-type A plus SCN compared to Fusarian solani-type A alone.

This study was conducted to determine the relationship between SCN resistance and SDS response in random segregating F₅- and F₆-derived lines.

Materials and Methods: Genetic material: Segregating lines from two crosses of SCN-susceptible parents by SCN-resistant parents were used in this study. One set of 89 lines was derived from 'Pyramid' (SCN race 3 and 14 res. and mod. res. to SDS) by 'Douglas' (SCN and SDS susc.). These segregating lines were randomly selected from agronomically acceptable plants in F₆ advanced by single seed descent (SSD) and the F_{6,8} generation was tested. The second set of 100 lines was derived from 'Forrest' (SCN race 3 res. and mod. res. to SDS) by 'Essex' (SCN and SDS susc.), derived in F₅ in the manner described above, and tested as F_{5,7} lines.

Field test: The random lines of both crosses were planted in a field in Pulaski County, IL, with a past history of SDS infestation and heavy SCN race 3 and possibly race 14 infestation. A lattice design with two replications was used. Data collected included disease incidence (DI) as a percentage of plants with visible leaf symptoms of SDS, disease severity (DS) on a scale of 1-9 with scores 1-5 describing increasing chlorosis and necrosis and scores 6-9 describing increasing defoliation and death of the plants, reproductive growth stages (R), and yield in kg/ha. DI, DS, and R scores were taken weekly from the onset of disease symptoms. The DI and DS ratings were linearly

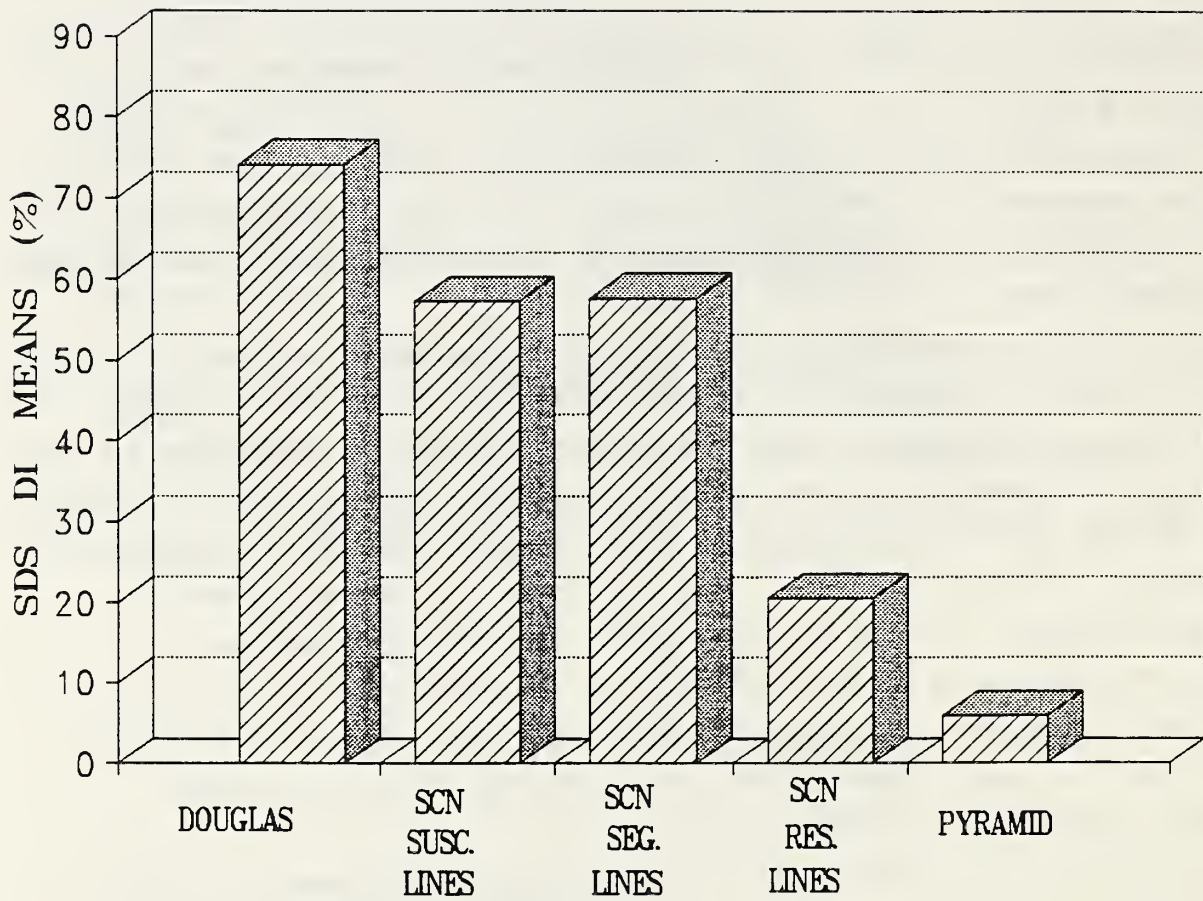


Fig. 1 DI for F6 derived lines of Pyramid x Douglas, grouped by response to SCN.

interpolated to a standardized R6 (full pod) score,

Greenhouse tests: The same random lines for both crosses that were field tested were screened for SCN response on a known race 3 soil in the greenhouse. The bare roots were rated 0-5 where 0=0 white females, 1=1-5 females, 2=6-10 females, 3=11-30 females, 4=31-60 females, and 5=>60 females. The lines were classed as resistant (all plants with SCN scores <3), segregating (mixed response), and susceptible (all plants with SCN scores >3) to SCN race 3. The actual cyst (white female) count on Essex ranged from 197 to 487 cysts/plant.

Results and Discussion: The proportion of SCN-resistant lines was higher than anticipated (Table 1). A possible explanation is that the SSD generations were grown on fields with mild SCN infestation, as was the generation in which individual plants were selected. The Pyramid x Douglas cross showed that the parents differed by 68 DI units (74 vs. 6%, Fig. 1), while the SCN-resistant and susceptible derived lines differed by 37 in DI (57 vs. 20%). Thus, 54% of the difference in DI between the parents was accounted for by SCN race 3 resistance. Also, 53% of the difference in DS between the parents was associated with SCN race 3 resistance. With the Essex x Forrest cross, the parents differed by 61 (81 vs. 20%) in DI and a 32 percentage point difference was found between SCN-resistant and susceptible lines (60 vs. 28%, Fig. 2). Therefore, 53% of the difference between parents was accounted for by SCN race 3 resistance. Additionally, 48% of the parental difference in DS for the Essex x Forrest cross was credited to the race 3 SCN resistance. A substantial portion of the DX score ($R6DI * R6DS/9$) was also attributed to SCN resistance (Table 1).

In lines from these two crosses, SCN race 3 resistance accounted for a sizable part of the difference in DI, DS, DX, and yield. However, roughly half of the difference appears to be due to unidentified genetic factors unrelated to SCN resistance. At least one year of additional data will be collected to determine the magnitude of heritability and of genotype x environment interactions.

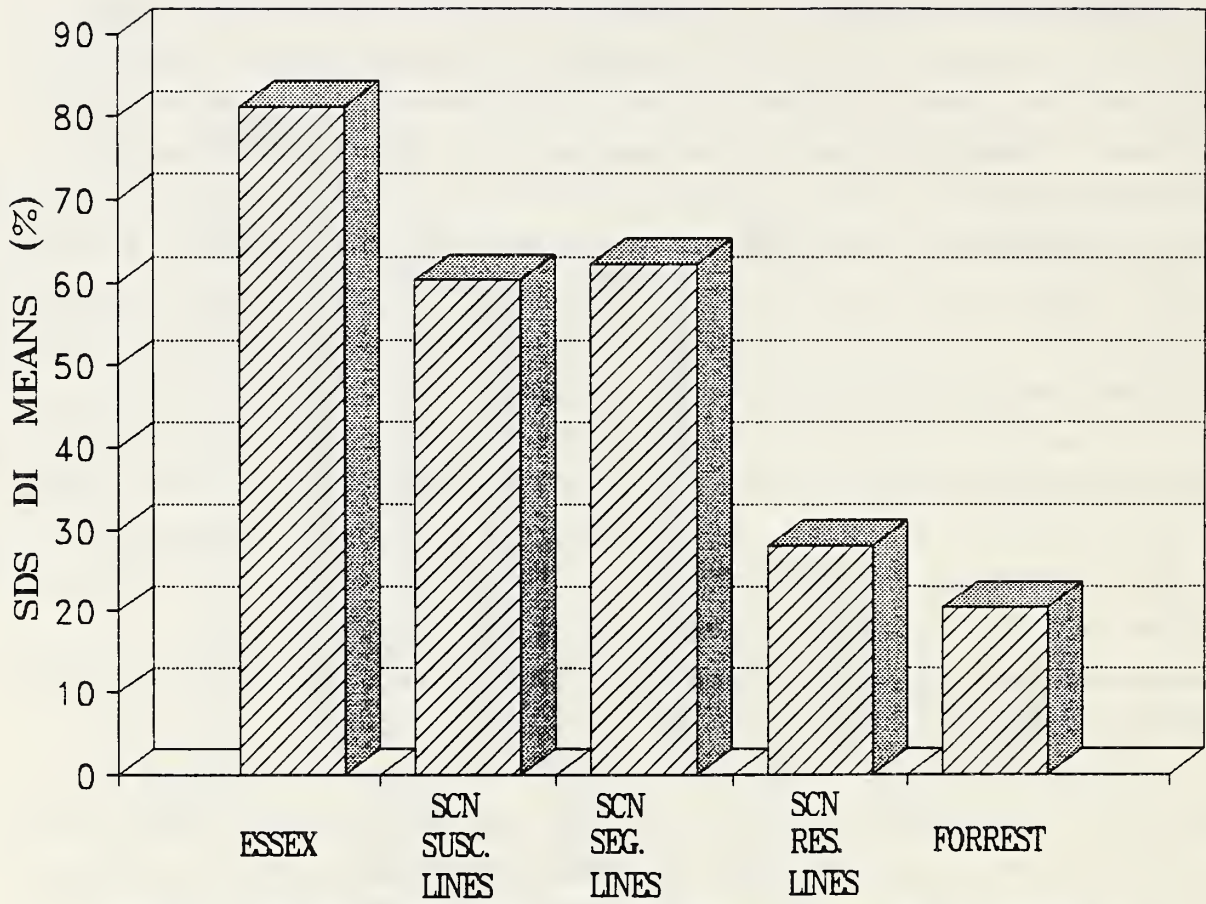


Fig. 2 DI for F5 derived lines of Essex x Forrest, grouped by response to SCN

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Table 1. SDS scores and yield for randomly derived, advanced generation soybean lines. DI=disease incidence; DS=disease severity; DX=disease index (described in text).

Pyramid x Douglas F6-derived lines					
SCN class	No. entries	DI	DS	DX	Yield
Douglas (susceptible)	4	73.9 a	2.2 ab	21.2 a	3040 abc
Susceptible lines	35	57.1 a	1.9 a	14.6 a	2845 c
Segregating lines	13	57.6 a	1.7 ab	12.3 a	3067 bc
Resistant lines	41	20.5 b	1.3 b	4.0 b	3295 ab
Pyramid (race 3, 14 resistant)	4	5.9 b	1.1 b	0.7 b	3383 a
Essex and Forrest F5-derived lines					
SCN class	No. entries	DI	DS	DX	Yield
Essex (susceptible)	8	81.2 a	2.1 a	19.6 a	3490 a
Susceptible lines	49	60.2 a	1.8 a	13.8 a	2979 cd
Segregating lines	28	62.1 a	1.8 a	14.2 a	3107 bc
Resistant lines	24	28.0 b	1.5 b	5.4 b	3167 b
Forrest (race 3 resistant)	8	20.4 b	1.4 b	3.3 b	2912 d

Means in a column followed by a common letter are not significantly different at $P=0.05$ by pairwise T-tests.

W. J. Matthews

V. N. Njiti

P. T. Gibson

M. A. Shenaut

UNIVERSITY OF KENTUCKY
 Department of Agronomy
 Lexington KY 40546

1) Test for linkage between lx_1 and ln , pc , f , i , r , dt_1 , lf_1 , and p_1 .

Lipoxygenases are a class of enzymes that catalyze the hydroperoxidation of polyunsaturated lipids such as linoleic acid and linolenic acid found in soybean seeds. Three soybean seed lipoxygenase isozymes have been characterized, and null alleles of each isozyme, inherited as simple recessive alleles, exist (Davies and Nielsen, 1986; Hildebrand and Hymowitz, 1982; Kitamura et al., 1983). Lipoxygenase-1 and lipoxygenase-2 are tightly linked, while lipoxygenase-3 is independent from these other two isozymes. Gene Lx_1 has been reported to be inherited independently of the genes Le , Sp_1 , and w_1 (Hildebrand and Hymowitz, 1982). The genotype L_1-5 (Davies and Nielsen, 1987) which has the recessive null allele lx_1 was crossed to several genetic stocks containing different marker alleles in order to determine linkage relationships. Seed chips of individual F_2 seeds were tested for their phenotype at the Lx_1 locus and the seeds separated into groups exhibiting the presence and absence of lipoxygenase-1. The F_2 seeds were planted and F_2 plants scored for the phenotypes of the other genes.

The table indicates that none of the genes was linked to the Lx_1 gene, as all pairs of genes segregated with a 9:3:3:1 phenotypic ratio. Even though segregation in the cross with the dt_1 marker genotype did not segregate 3:1 at the Lx_1 gene, there was no indication of linkage with dt_1 , within each Lx_1 phenotype ($lx_1\ lx_1\ Dt_1\ __$: $lx_1\ lx_1\ dt_1\ dt_1$, obs. 30:12, exp. 31.5:10.5; $Lx_1\ __ Dt_1\ __$: $Lx_1\ __ dt_1\ dt_1$, obs. 55:22, exp. 57.75:19.25).

Table 1. Number of individuals of each phenotype.

Gene# tested	A [^] —		aa [^]		X ^{2*} 3:1	X ^{2*} 3:1	X ^{2**} 9:3:3:1
	<u>Lx</u> ₁ —	<u>lx</u> ₁ —	<u>Lx</u> ₁ —	<u>lx</u> ₁ <u>lx</u> ₁	<u>Lx</u> ₁	A ⁺	
<u>P</u> ₁	58	25	17	11	3.27	0.05	4.23
<u>i</u>	63	25	17	7	0.76	0.76	1.52
<u>r</u>	64	25	16	7	0.76	1.19	1.95
<u>pc</u>	115	28	28	10	1.55	1.55	3.94
<u>f</u>	107	90	33	8	1.26	0.37	1.69
<u>ln</u>	164	60	51	16	0.19	0.60	1.03
<u>Lf</u> ₁	100	20	31	12	2.50	0.16	4.73
<u>dt</u> ₁	55	30	22	12	6.72	0.81	---

[^] A — denotes the dominant phenotype and aa denotes the recessive phenotype of any of the genes tested for linkage with gene Lx₁.

⁺ A denotes any of the genes tested for linkage with Lx₁.

The allele listed for the gene tested is the allele present in the marker genotype that was crossed to L₁ - 5.

* X² (1,0.05) = 3.84

** X² (3,0.05) = 7.81

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T. W. Pfeiffer

D. F. Hildebrand

2 Using a possible allozyme of lipoxygenase-1 to select for the linked allele lx_2 in the heterozygous state during backcrossing.

Lipoxygenases catalyze the hydroperoxidation of polyunsaturated fatty acids such as linoleic acid found in soybean seeds; the linoleic acid hydroperoxides are subsequently converted to volatile compounds such as hexanal which are associated with undesirable flavors in processed soybean protein products. Three soybean seed lipoxygenase isozymes have been characterized, and null alleles of all three isozymes have been identified in the USDA germplasm collection (Hildebrand and Hymowitz, 1981; Kitamura et al., 1983). The lipoxygenase-1 and lipoxygenase-2 genes are very tightly linked in repulsion, and it has not been possible to produce a genotype with the recessive null alleles homozygous at both loci (Davies and Nielsen, 1986). Davies and Nielsen (1987) developed backcross-derived near-isogenic lines of 'Century' containing the different lipoxygenase null alleles. Davies et al. (1987) concluded that elimination of lipoxygenase-2, through the use of the lx_2 allele, was important in improving the flavor of soybean food preparations, and soybean breeders have started to utilize the L_2 -3 Century isolines as a source of the lx_2 allele for backcrossing.

Recently we have become aware that the lipoxygenase-1 enzyme present in lx_2lx_2 seeds is an electrophoretic variant of the lipoxygenase-1 enzyme produced by many soybean genotypes, including Century, which are homozygous for the Lx_2 allele. The lipoxygenase-1 found in Century as a pI of 5.85, and the variant found in lx_2lx_2 genotypes has a pI of 5.79. This variant has been carried through the backcrossing generations into L_2 -3. Our information supports the previous report that the lipoxygenase-1 and lipoxygenase-2 genes are very tightly linked; no genetic recombinants were found from the analysis of approximately 200 F_2 seeds from the cross L_2 -5 (lx_2lx_2 Lx_2Lx_2) \times L_2 -3 (Lx_2Lx_2 lx_2lx_2). Also, in the F_2 progeny of the cross Century \times L_2 -3, we have not detected any variation in the association of the lipoxygenase-1 electrophoretic variant with the lx_2 lx_2 genotype. In the F_1 seeds of this cross, as well as in the F_1 seeds of crosses between L_2 -3 and other parents with the Century type lipoxygenase-1, two electrophoretic bands representing both lipoxygenase-1 types are seen.

The presence of two electrophoretic bands for lipoxygenase-1 when gene

Lx₂ is in a known heterozygous state, coupled with the very tight linkage between lipooxygenase-1 and lipooxygenase-2 genes, makes it possible to identify Lx₂ Lx₂ genotypes during backcrossing of the Lx₂ allele by electrophoretic analysis of lipooxygenase-1. Thus, one can eliminate the selfing generation during backcrossing and shorten the time required to produce a backcross-derived variety. Details of the electrophoresis method used to identify the lipooxygenase-1 electrophoretic variant are available in Grayburn et al. (1991).

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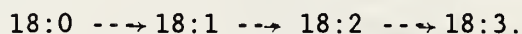
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T. W. Pfeiffer

D. F. Hildebrand

3) Results from crosses of low linolenate soybean mutants.

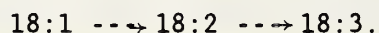
Linolenic acid (18:3) is synthesized by consecutive desaturations of stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2):



The low linolenate mutant designated as A5 described by Hammond and Fehr (1984) is low in both 18:2 and 18:3 and high in 18:1. A5, therefore, would appear to have reduced desaturation from 18:1 to 18:2. The low 18:3 mutant C1640 described by Wilcox et al. (1984), on the other hand, is low in 18:3 and high in 18:2 in the field and greenhouse environment of Lexington KY (Wang et al., 1989). C1640 appears to have a reduction in 18:2 to 18:3. These results suggest the following model of 18:3 biosynthesis in seeds of these soybean genotypes:

Century	18:1	→	18:2	→	18:3
A 5	18:1	→	18:2	→	18:3
C1640	18:1	→	18:2	→	18:3

An F_2 might be expected from crosses of A5 and C1640 with reduced desaturation of both 18:1 and 18:2 :



This might have a further overall reduction in the biosynthesis of 18:3 resulting in a transgressive segregant with lower 18:3 than A5 or C1640. To this end, we analyzed F_2 seed from 113 F_2 plants from the cross A5 x C1640 and seed from 30 plants of each parent grown in the field in Lexington KY in 1989 for fatty acid composition.

Genotype	18:3/18:2		18:1/18:2		18:3/18:1	
	High	Low	High	Low	High	Low
A5	0.16	0.07	1.61	0.47	0.27	0.07
C1640	0.11	0.05	0.41	0.20	0.58	0.17
F_2	0.13	0.07	1.30	0.25	0.41	0.11

These data again show clear differences between A5 and C1640 in terms of unsaturated fatty acid ratios particularly with regard to 18:1. Apparently, the combination of the reduced desaturation from 18:1 to 18:2 from A5 and 18:2 to 18:3 from C1640 did not result in any transgressive segregants with further reductions in 18:3. This is consistent with the report of Hammond and Fehr (1984) involving crosses between A5 and their low 18:3 Century mutant. A maternal influence on expression of altered fatty acid composition is seen in crosses involving A5 (Graef et al., 1988) and also for 18:1 in crosses involving C1640 (Wilcox and Cavins, 1985). Low 18:3, however, shows no maternal influence in crosses with C1640 (Wilcox and Cavins, 1985). Because of the maternal influence on 18:1, we sampled F_3 seed from F_2 plants rather than F_2 seed. The lack of transgressive segregants for low 18:3 from this cross involving two low 18:3 mutants is probably due to a strong influence of genetic background. The inheritance of low 18:3 from crosses involving A5 is complex (Graef et al., 1988) and the effect on the 18:1 to 18:2 step is probably indirect rather than an altered 18:1 desaturase. Additional soybean mutants will likely be needed with specific alterations in this desaturation step for ready genetic alterations of unsaturated fatty acids in soybeans.

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David F. Hildebrand

Todd Pfeiffer

4) Nondestructive rapid screening technique for lipoxygenase 1 in soybean seeds.

Lipoxygenases are a group of enzymes that catalyze the peroxidation of polyunsaturated fatty acids and derivatives. This can lead to a number of primary and secondary oxidation products that are of importance in food (and feed?) quality and may contribute to the oxidative deterioration of seeds in storage. Lipoxygenases normally exist in soybean seeds as three forms or isozymes that represent different gene products. Null mutants have been found for all three soybean lipoxygenase isozymes and these have been used for breeding and genetic studies. The standard procedure that has been employed to identify null mutant seeds for one or more of the lipoxygenase isozymes utilizes electrophoretic gels. In this note we describe a more rapid procedure for screening for the presence or absence of lipoxygenase 1 activity that should be more amenable to routine breeding and genetic programs.

This procedure does not require that the proteins be extracted from the soybean seed prior to analysis. Rather, enzyme is extracted from thin slices of individual soybean seeds concomitant with the reaction with the test solution A, which provides the substrate for lipoxygenase. This procedure is nondestructive.

Solutions: Solution A: 0.08% Tween 20, 40 mM potassium borate, pH 9.0, 1 mM linoleic acid. Solution B: 5 ml saturated aqueous KI per 100 ml 15% acetic acid (Guss et al., 1967). Solution C: 1% soluble starch.

Procedure: 1) Cut a small chip or slice a few shavings from the cotyledons of the soybean seed end that does not contain the embryonic axis. The seed coat does not contain detectable lipoxygenase 1 and will not change the test by its presence or absence. Large chips give ambiguous results and so should be avoided. The chips can be kept several days. Place seed embryo end into a length of plastic tubing to hold seed and shave the seed's end with a scalpel or razor blade onto weighing wax paper. Pour the chips/shavings into a test tube (e.g., 12 x 75 mm).

2) Add approximately 1 ml solution A (substrate) to each tube. Large numbers of 50 to several hundred are easily done in groups. Wait 10 minutes before adding one drop or 50 ul of solution B (acidic KI). One drop, or 50 ul, solution C (soluble starch) is added last. No wait is required between adding solution B and solution C.

Soybean seeds that are heterozygous or homozygous dominant for lipooxygenase 1 will produce a purple color with this test. Tests of homozygous recessive seeds that are null for lipooxygenase 1 remain milky white. Some soybean lines will show greater enzyme activity, thus producing a deeper purple color more quickly. In most cases, the color fades in 20 to 30 hours - earlier on the quicker color-producing samples. Tubes should be read at 4 hours and 20 hours. The 4-hour reading catches most positives (purples) some of which turn clear in 20 hours. The 20-hour reading catches positives that had not yet turned purple at 4 hours. The more enzyme-active segregating populations will have some seed samples that quickly produce dark purples (in 2 hours) and some that more slowly produce pale purples. These may represent homozygous and heterozygous seeds, respectively.

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David F. Hildebrand

Eugene C. Lacefield

Todd Pfeiffer

FORT VALLEY STATE COLLEGE
Agricultural Research Station
Fort Valley GA 31030-3298

1) Soybean harvest index as related to yield, plant architecture and economic value.

The universal intent of agricultural scientists to produce maximum yields from minimum input mandates study of biological efficiency of crop plants. In soybean and other crops, harvest index has been used as a measure of biological efficiency since it takes into account both the total dry matter production and economic yield (Schapaugh and Wilcox, 1980; Bhardwaj and Bhagsari, 1989, 1990; Joshi et al., 1989). The importance of harvest index in cereals was demonstrated by Singh and Stoskopf (1971). Donald and Hamblin (1976) concluded that harvest index can be an effective criterion for yield evaluation and improvement. We had previously presented data indicating existence of significant variation in soybean harvest index and its relationship to yield, leaf area, phytomass, and plant height (Bhardwaj and Bhagsari, 1989, 1990). The present study was conducted to evaluate the relationship of soybean harvest index with yield, plant architecture (pods/plant and seeds/pod) and economic value (oil and protein content.)

Materials and Methods: Fifteen genotypes (Table 1) were planted in 4-row plots in a randomized complete block design with four replications on May 25, 1989. The rows were 4.5 m long and 0.9 m apart. A 0.3-m long sample was harvested from each plot at final harvest (Oct. 5-Nov. 14, 1989) to record the total number of plants, pods, and seeds. The pods/plant and seeds/pod were calculated. The yield (kg/ha) and harvest index (%) data were calculated from another 1-m long sample from each plot. The above ground plant material was dried to a constant weight and its weight was recorded before thrashing with a plot combine. After thrashing, the seed weight was recorded and used for yield calculations. The harvest index was calculated as seed weight/weight of dried above-ground plant material and expressed as percentage. The leaves and petioles had fallen off by this time and the total above-ground material consisted of seeds, stems, and branches. The oil and protein contents were determined from a composite sample of each genotype, at Northern Regional Research Laboratory, Peoria IL.

Results and Discussion: Significant variation existed among the genotypes for harvest index, yield, pods/plant, and seeds/pod (Table 1). The harvest index (%) varied from 34.1 (D71-V89) to 47.4 (TN5-85). The highest yielding genotype was G80-1413, which has been released by Georgia Agricultural Experiment Station as "Thomas". The lowest yield was recorded for 'Rocky', small-seeded genotype. Pods/plant varied from 51 (G81-152) to 141 (TN4-86) whereas seeds/pod varied from 1.4 (Tokyo) to 2.7 (G81-152).

Harvest index was positively correlated with pods/plant ($r = 0.35^{**}$) and seeds/pod ($r = 0.32^{**}$). Seed yield was not related to harvest index, pods/plant or seeds/pod. Pods/plant for the five genotypes classified as highly efficient (based on their high harvest index) as a group had significantly more pods/plant as compared to the five genotypes with low biological efficiency. These groups did not differ for seeds/pod.

Oil content (%) varied from 16.7 (D71-B86) to 21.2 (TN4-86) whereas the protein content (%) varied from 41.9 (G81-152) to 49.0 (D71-V86). Harvest index, pods/plant, and seeds/pod were not related to oil or protein content but yield exhibited a significant negative correlation with protein content ($r = 0.61^{*}$). A negative relationship also existed between protein and oil content ($r = 0.57^{*}$).

These results indicate that it might be possible to improve harvest index by increasing the number of pods/plant. The absence of significant correlation between harvest index and economic value (oil and protein content) indicates that high biological efficiency can be achieved without affecting oil or protein content.

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Table 1. Characteristics of soybean genotypes during 1989 at Fort Valley GA.

Genotype	Harvest index (%)	Yield kg/ha	Pods/ plant	Seeds/ pod	Oil (%)	Protein (%)
TN5-85	47.44	2.33	82.81	1.97	45.8	20.3
TN4-86	45.56	2.44	141.06	2.49	45.7	21.2
EPPS	45.15	2.39	91.90	2.25	46.0	19.9
G80-1413	44.24	3.26	83.82	1.91	46.0	19.1
ROCKY	43.25	1.75	118.14	1.51	46.4	17.0
VANCE	43.02	2.01	74.89	2.31	45.2	18.8
GORDON	42.03	3.24	84.40	1.88	43.5	18.0
SC82-1672	41.00	3.91	96.36	1.90	43.6	19.6
G81-234	39.55	3.98	60.63	2.29	43.1	19.8
LATE-GIANT	38.35	2.86	66.90	1.43	42.8	20.7
D71-V86	38.32	2.10	54.13	1.53	49.0	16.7
TOKYO	38.11	3.30	78.02	1.39	45.5	18.5
G81-152	37.97	2.93	51.09	2.70	41.9	19.7
TANBAGURA	35.84	2.92	61.65	1.55	43.7	21.0
D71-V89	34.12	2.40	51.39	1.45	47.4	17.6
Mean	40.92	2.78	79.81	1.91	45.0	19.2
LSD (0.05)	4.05	0.64	48.47	0.41	-	-

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H. L. Bhardwaj

A. S. Bhagsari

UNIVERSITY OF MISSOURI
Delta Center
Portageville MO 63873

1) Release of cyst nematode-resistant soybean germplasm.

Soybean germplasm S88-2036. S88-2036, a soybean cyst nematode (SCN) Heterodera glycines resistant germplasm line of soybeans, was released in March, 1990, by the Missouri Agricultural Experiment Station for breeding and experimental purposes. S88-2036 is resistant to all known races and biotypes of SCN in the United States. S88-2036 derived its SCN resistance from PI 437,654, a plant introduction that was reported to be resistant to Races 1 through 5 (Anand et al., 1988). In subsequent testing we found PI 437,654 to be resistant to all races and biotypes of SCN collected from different regions of the US.

S88-2036 was developed at the Delta Center of the University of Missouri, Portageville MO. It originated from the cross PI 437,654 x Forrest³ using backcross method of breeding. The F₂ plants in each cross were screened in the greenhouse against a gene pool of SCN races. The gene pool of SCN consisted of a mixture of races 1, 2, 3, 4, 5, 6, 9, and 14, along with a few undefined biotypes of SCN. These were collected from soybean fields in Missouri, Arkansas, Tennessee, North Carolina, Georgia, Florida, Iowa and Ohio. Five resistant F₃ plant progenies with a maximum of two cysts per plant were selected. From each progeny 75 to 100 plants were harvested and yield evaluated in a progeny row test in the F₄ generation. Ten plants from each of the high yielding progenies were saved for SCN screening and the rest of the seed was increased in Puerto Rico. Selected progenies were yield-tested in replicated tests at three locations representing clay, loam, and sandy soils in southeast Missouri in 1989. Among these, S88-2036 was selected for its superior performance and high level of resistance to SCN. It yielded as good or slightly better than 'Forrest' and 'Bedford' in clay and loam soils and was significantly superior to these two cultivars in SCN-infested sandy soils.

S88-2036 has determinate plant type and is in maturity group V, similar to Forrest. It has white flowers and tawny pubescence. It is slightly shorter in plant height and about two days earlier than Forrest. The seeds are yellow with black hila.

S88-2036 has a potential to be grown as a cultivar. At present, it is

being released to provide a useful source of germplasm to develop varieties resistant to a large number of SCN races. Seeds of S88-2036 will be provided upon written request and agreement to acknowledge the source if the germplasm contributes to a cultivar or a new germplasm.

Soybean population S89-3151. A soybean population (S89-3151) which was developed by intermating nine different plant introductions (PI's) has been released for research and developmental purposes. Nine PI lines, 'Peking', PI 88,788, PI 90,763, PI 437,655, PI 89,778, PI 404,166, PI 209,332, PI 438,503A, and PI 437,654, were selected on the basis of their diverse origin and resistance to at least two SCN races. These PI lines were crossed with a genetic male sterile line of 'Ogden'. Generations of intermating alternated in the generations of selfing, in which F_2 population was always planted in isolation and bordered by a mixture of resistant plants. Following the third cycle of intermating, the F_2 plants were screened for reaction to SCN races, and resistant progenies were bulked. The F_3 generation is expected to segregate in the ratio of five male fertile : 1 male sterile.

S89-3151 has both determinate and indeterminate plants with different seed coat color. The plants may vary from maturity group III through VI. About 500 seeds of S89-3151 will be distributed free of charge on request as long as the supply lasts. There will be no restriction on the use of the material except to acknowledge the source if the germplasm contributes to the development of a cultivar or a new germplasm.

Breeder seed of S88-2036 will be maintained by Dr. S. C. Anand, Department of Agronomy, University of Missouri, Delta Center, Portageville MO 63873.

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UNIVERSITY OF CENTRAL OKLAHOMA

Department of Biology

100 North University Drive

Edmond, OKLAHOMA 73034

and

IOWA STATE UNIVERSITY

Department of Agronomy

Ames, IOWA 50011

1) Subcellular enzyme activities of mutant soybean lines lacking mitochondrial MDH isozyme bands

Mutable-variation investigations have recently enabled alteration of isozyme banding patterns for malate dehydrogenase (MDH) in soybean (Amberger, 1990; Hedges, 1989). These variants demonstrate banding patterns lacking mitochondrial MDH (mMDH) in the cultivar lines w4-m (from Asgrow mutable) (Hedges, 1989), y20-k2 (from Harosoy) (Hedges, 1989), and Jilin 3 (from Jilin 3) (Amberger, 1990). What is surprising about these results is that, although the isozymic mutations appear to be mitochondrial, phenotypic variations exhibit low levels of chlorophyll. From these results it is apparent that some mutable mechanism is responsible for regulating mMDH expression and chlorophyll synthesis in chloroplasts of the variant lines, w4-m, y20-k2, and Jilin 3. Whether this mechanism is physiological, genetic, or a combination thereof has yet to be determined. Research proposed in this investigation will provide information on mMDH activity needed to better understand the physiological mechanisms underlying mutations observed.

Malate dehydrogenase demonstrates variability in isozyme pattern (Hedges, 1989; Amberger, 1990), genetic inheritance (Goodman et al., 1981), and physiological function (Palmer, 1976). Although some MDH enzymes exist in the cytosol and chloroplast of plants, this research focuses on mMDH enzymes because variants found in soybeans lack bands for the mitochondrial form of the enzyme (Hedges, 1989). In mitochondria, the metabolic function of MDH is conversion of MDH to oxaloacetate (OAA) coupled to NADH production.

Physiological explanation for mMDH-imposed chlorophyll deficiencies has potential, although genetic differences probably exist for specific MDH alleles and may exist for loci encoding chlorophyll synthesis. Two scenarios

have been developed to suggest possible mechanisms relating the MDH mutation to chlorophyll deficiency. The first scenario associates decreased MDH activity and deficient malate and glutamate transport with degenerated chlorophyll synthesis. A second scenario hypothesizes a transposable element that may alter genetic structure of associated genes for mMDH and chlorophyll synthesis.

The first scenario involves interrelationships between MDH, malate, glutamate, and chlorophyll in the mitochondrion, glyoxysome, cytosol, and chloroplast. In this mechanism, retarded mitochondrial and glyoxysomal MDH activity results in a lack of OAA and succinate by means of the TCA cycle and glyoxylate pathway. Subsequently, lack of OAA and succinate causes a deficiency in α -ketoglutarate via the TCA cycle and shuttle mechanisms. Deficient α -ketoglutarate causes a lack of glutamate and hence, lack of glutamate results in retarded chlorophyll synthesis. In addition to causing lack of the glutamate template for chlorophyll synthesis, retarded mMDH activity may also curtail cytosolic and chloroplast-reducing equivalents (in the form of malate) needed for the synthesis of chlorophyll.

In the second scenario, a transposable element alters MDH activity and chlorophyll synthesis through insertion or modified deletion after transposition. Retarded chlorophyll synthesis may be coincident with transposon-induced deactivation of the mMDH gene. Recurring transposition leads to chromosomal deletion, inversion, duplication, and recombination which can curtail mMDH expression as well as expression of other genes. It is evident from this hypothesis that a transposon inhibiting mMDH expression could lead to yellow plants if the transposon coincidentally affects chlorophyll synthesis.

While both scenarios provide explanation of chlorophyll differences in MDH soybeans, other speculative mechanisms may integrate their reasoning into one hypothesis. Before such a hypothesis is devised, it will be necessary to obtain more information on the enzyme characteristics, chlorophyll quality and content, heritability, and genetic sequence of normal and mutant soybean plants.

Materials and Methods: Nine soybean lines representing five mutant (malate-deficient and chlorophyll-abnormal) and four normal plants were used for this investigation. These lines were grouped as follows based on pedigree:

Harosoy (wild)	Jilin 3 (wild)	Asgrow mutable (<u>w4-m</u>)
Harosoy (<u>k2</u>)	Jilin 3 (mutant)	CD-1 (mutant)
Harosoy (<u>y20-k2</u> mutant)		CD-2 (mutant)
		CD-3 (mutant)

These homozygous lines were obtained from R. G. Palmer, USDA-ARS, Iowa State University, Ames IA. A total of 20 seeds from each of the nine lines were used for germination. Seeds were germinated in a randomized complete block design with four replicates of five seeds with cultivar as the whole plot and mutation as the sub plot.

Cotyledonary tissue from seven-day-old seedlings was ground in grinding buffer and centrifuged at different *g* forces and through different concentrations of sucrose to obtain crude chloroplast and "washed" mitochondrial fractions according to procedures modified after Obenland (1989), Day and Hanson (1977), and Douce et al. (1972) as outlined by Bidlack and Bidlack (unpublished). Protein and MDH assays of these fractions were performed spectrophotometrically (J. Bidlack, unpublished).

Purity of each extract was evaluated by light microscopy and isozyme analyses (Cardy and Beversdorf, 1984). Observation of chloroplasts by light microscopy and presence of chloroplast bands (Bidlack and Bidlack, unpublished) in starch gels were used to confirm purity of chloroplast preparations. Mitochondrial purity was determined by presence of mitochondrial isozyme bands (Amberger, 1990; Hedges, 1989) in starch gels.

Data were evaluated by PROC GLM to obtain standard deviations for enzyme activities in units of $\mu\text{mol/g protein/min}$.

Results and Discussion: Microscopic evaluation and isozyme analyses indicated good isolation of chloroplasts and excellent isolation of mitochondria, although some mitochondrial contamination was noted (by isozyme analysis) in chloroplast extracts. Although chloroplast isolation did not appear completely "pure," relative contaminants were uniform across samples and probably did not contribute towards normal and mutant enzymatic differences. Since the focus of this research was on mMDH activity differences, excellent isolation of mitochondria, confirmed by isozyme analysis, persuaded continuation of the experiment.

In each set of near-isogenic lines, mitochondrial MDH activity was lower in mutant seedling extracts compared to those of the normal (Tables 1-3).

This was especially true in the case of Harosoy (Table 1) and Asgrow (Table 3) lines, in which the mutant demonstrated significantly lower mMDH activity compared to the normal. In all cases, chloroplast MDH activity of mutant seedling extracts was either the same or higher than those of the normal (Tables 1-3).

Table 1. Chloroplast and mitochondrial MDH activity (\pm SD) of wild type and mutant near-isogenic lines of Harosoy.

Grouping	Activity	
	----- $\mu\text{mol g protein}^{-1} \text{ min}^{-1}$ -----	
	Chloroplast	Mitochondrial
Harosoy (wild type)	65.4 \pm 1	179.8 \pm 47
Harosoy (<u>k2</u>)	147.6 \pm 8	135.1 \pm 2
Harosoy (<u>y20-k2</u> mutant)	100.0 \pm 23	68.5 \pm 5

Table 2. Chloroplast and mitochondrial MDH activity (\pm SD) of wild type and mutant near-isogenic lines of Jilin.

Grouping	Activity	
	----- $\mu\text{mol g protein}^{-1} \text{ min}^{-1}$ -----	
	Chloroplast	Mitochondrial
Jilin 3 (wild type)	55.5 \pm 13	114.4 \pm 47
Jilin 3 (mutant)	69.7 \pm 7	71.3 \pm 15

Table 3. Chloroplast and mitochondrial MDH activity (\pm SD) of wild type and mutant near-isogenic lines of near-isogenic lines of Asgrow.

Grouping	Activity	
	----- $\mu\text{mol g protein}^{-1} \text{ min}^{-1}$ -----	
	Chloroplast	Mitochondrial
Asgrow mutable (<u>w4-m</u>)	82.9 \pm 26	113.4 \pm 17
CD-3 (mutant)	91.0 \pm 55	96.1 \pm 21
CD-2 (mutant)	78.7 \pm 9	77.5 \pm 30
CD-1 (mutant)	73.5 \pm 7	68.3 \pm 16

Significantly lower mMDH activities in mutant seedling extracts compared to those of the normal indicates a deficiency in mitochondrial function in mutant plants. This supports original findings by Hedges (1989) and Amberger (1990) who reported missing mitochondrial bands in isozymic analyses of mutant lines derived from Harosoy, Jilin 3, and Asgrow mutable (w4-m). Since these mutant lines phenotypically demonstrate chlorophyll-deficient leaves and since mutant chloroplast MDH activity does not appear to be significantly different

from that of the normal, it is possible that mitochondrial MDH alterations could lead to chlorophyll abnormalities. Whether or not this mechanism is physiological, genetic, or a combination thereof has yet to be determined.

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James E. Bidlack

Laurie A. Bidlack

William Caire

University of Central Oklahoma

Reid G. Palmer

Randy C. Shoemaker

USDA-ARS Iowa State University

IOWA STATE UNIVERSITY

Department of Food Science and Human Nutrition

Department of Agronomy

Ames IA 50022

1) Rapid test for lipoxygenase-2 and -3.

Lipoxygenases are a group of enzymes that catalyze the peroxidation of polyunsaturated acyl groups in damaged plant tissue. Lipoxygenase may contribute to the oxidative deterioration of seed and to the development of beany off-flavors in soybean meal. Lipoxygenase oxidation products produced during the extraction of soybean meal may reduce the stability of refined oil (Frankel et al., 1988).

Lipoxygenases normally exist in soybean seeds as three isozymes that represent different gene products (Shibata et al., 1987 and 1988; Yenofsky et al., 1988). Null mutants have been found for three soybean lipoxygenase isozymes (Davies and Nielsen, 1986; Hildebrand and Hymowitz, 1982; Kitamura et al., 1983). Lipoxygenase-1 seems less important in generating oxidized flavors than lipoxygenase-2 and -3 (Frankel et al., 1988; Davies and Nielsen, 1987), but this depends on the processing conditions (Zhuang et al., 1991). Recent studies indicate that lipoxygenase-2 and -3 also differ in their impact on C₆-aldehyde formation (Hildebrand et al., 1990).

Gel electrophoresis and immunological tests have been used to identify seed with null alleles for one or more of the lipoxygenase isozymes. We have developed a more rapid procedure for screening for the presence or absence of lipoxygenase-2 and -3 isozymes. These methods do not require that the proteins be extracted from the soybean seed before analysis, and they are suitable for routine breeding and genetic programs.

Solutions: Solution A: 10 g refined soybean oil blended in 100 ml of water containing 1.25 g of gum arabic. Solution B: Freshly prepared solution of 4 g ferrous sulfate and 4 g ammonium thiocyanate in 100 ml of water.

Procedure: The soybean seeds to be analyzed for lipoxygenase-2 are crushed between two aluminum plates. These plates are rectangular, 22 cm by 23 cm and are 1.3 cm thick. One of the plates has circular indentations that are 2 mm deep arranged in a rectangular array 1.4 cm apart. A seed is placed in each of the indentations, the second aluminum plate is placed on top of the seeds, and the seed samples are crushed in a hydraulic press at 1500 to 3000 kg/cm.

The crushed seeds are left on the plate, and three to five drops of solution A are added to each seed. When all of the seeds have been treated, a sheet of Whatman No. 1 filter paper is pressed onto the wetted seeds so that moist spots are transferred to the paper corresponding to each of the wet seeds. Any seed pieces adhering to the paper are brushed off, and the paper is allowed to dry until most of the water has evaporated but the spots are still slightly moist. The paper is sprayed lightly with solution B and dried with hot air from a hair dryer. The spots from seeds with lipoxxygenase-2 will turn dark red while those lacking the isozyme will remain cream colored. The colors should be recorded as soon as they are apparent because all the spots will turn dark red in 0.5 to 1 hr. It is helpful to run control seeds with and without lipoxxygenase-2 on each test paper.

A similar color test may be used to test for lipoxxygenase-3 if lipoxxygenase-2 is absent, but a few drops of water are added to the crushed beans and they are allowed to soak in the water for 15 min. Sufficient water is added to the beans so that free water may be observed on the bean surface. Additional drops of water may be added during the 15-min wait to maintain free water on the surface. A paper is applied to the moist beans, and water from the beans is transferred onto the paper. One drop of solution A is added to the moist spot representing each bean. The paper is covered with plastic film to keep it from drying out and held for 25 to 30 min. Then the paper is sprayed with solution B and dried slowly with a hair dryer on low heat. About 20 to 30 min after spraying, the color is observed. Beans with lipoxxygenase-2 will be dark red, those without lipoxxygenase-2 and with lipoxxygenase-3 will be light pink, those without either lipoxxygenase-2 or -3 will be light yellow.

The tests have been verified using seed known to be null in the appropriate lipoxxygenase isozyme.

These tests depend upon the oxidation of polyunsaturated fatty acids in the soybean oil to hydroperoxides by the lipoxxygenase isozymes. The hydroperoxides oxidize ferrous ion to ferric ion, and ferric ion forms an intense red color with thiocyanate. This test is so sensitive for ferric ion that generally there will be considerable red color in the ferrous thiocyanate solution because of low ferric ion concentrations, but the color is insufficient to interfere with the evaluation.

There generally is much more reactivity of lipoxxygenase-2 than -3 in soybeans, so the former must be absent to test for the latter, and more time must

be allowed for the enzymic oxidation to occur. The test conditions for seed null in both lipoxygenase-2 and -3 can also be used to identify seed null only in lipoxygenase-2.

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- Bradner, H.R., King Agro. Inc., Box 1088, Chatham, Ontario Canada N7M 5L6.
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- Brown, Eddie, Hartz Seed Co., Box 946, Stuttgart AR 72160.
- Bubeck, David, Crop Sci. Dept., NCSU, Box 7620, Raleigh NC 27695.
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- Chandlee, Joel M., Dept. of Plant Sci., URI, 329 Woodward Hall, Kingston RI 02881.
- Cianzio, Silvia, Subestacion de Isabela, Aprtdo 506, Isabela PR 00662.
- CIBA-Geigy Corp., Biotech Research., PO Box 12257, Research Park Triangle NC 27709.
- Cooper, Richard W., Dept. of Agronomy, Ohio Agric. Res. & Dev. Ctr., Wooster OH 44691.
- Cregan, P., Soybean & Alfalfa Res. Lab., Range 1, HH 19, BARC-West, Beltsville MD 20705.
- Crook, Wayne, Pioneer Hi-Bred Intl., Box 627, Marshall MO 65340.
- Danna, Kathleen, Campus Box 347-MCDB, Univ. of Colorado, Boulder CO 80309-0347.
- Davey, M.R., Dept. of Botany, Univ. of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom.
- Devine, T.E., PMB Lab., Rm. 118, Bldg. 006, USDA BARC-West, Beltsville MD 20705.
- Ding, An-Lin, Inst. Crop Breeding & Cultivation, Chinese Acad., Agric. Sci., Beijing 100081, People's Republic of China.
- Dougherty, Richard, Hartz Seed Co., PO Box 946, Stuttgart AR 72160.

- Drzycimski, Deborah A., Asgrow Seed Co., 634 E. Lincolnway, Ames IA 50010.
 Eathington, Sam, W307 Furner Hall, 1102 S. Goodwin Ave., Urbana IL 61801.
 Eby, William, Midwest Oilseeds Inc., Rt. 3, Box 204, Adel IA 50003.
 Elden, T.C., USDA-ARS, Bldg. 467, BARC-East, Beltsville MD 20705.
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 Frank, Scott, Soybean Res. Foundation, 115 N. Perry St., Mason City IL 62664.
 Freestone, Robert, Pioneer Hi-Bred Intl., Inc., 3261 W. Airline Hwy., Waterloo IA 50703.
 Gage, Howard L., Northrup King Co., Hwy 158E, PO Box 729, Bay AR 72411.
 Gai Junyi, Agronomy Dept., Nanjing Agric. Coll., Jiangsu Province, Nanjing, People's Republic of China.
 Garland, Marshall L., Callahan Enterprises, 1122 E. 169th St., Westfield IN 46074.
 Grabau, Elizabeth, Dept. of Agron. and Plant Gen., Univ. of Minnesota, St. Paul MN 55108.
 Graef, George, 319 Keim Hall, Univ. of Nebraska-Lincoln, Lincoln NE 68583-0915.
 Gresshoff, P.M., Plant Mol. Genet (OHL), 269 Ellington Plant Sci. Bldg., Knoxville TN 37701-1071.
 Gritton, Earl T., Dept. of Agron., Univ. of Wisconsin, Rm. 442 Moore Hall, Madison WI 53706.
 Hackney, Jay, Prograin Seeds, Inc., 165 Senneville Rd., Senneville, Quebec Canada H9X 3L2.
 Hartman, Kerrick, Hartz Seed Co., PO Box 946, Stuttgart AR 72160.
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 Hokkaido Natl. Agric. Exp. Stn., Memuro, Hokkaido 082 Japan.
 Hicks, John D. Jr., Pioneer Hi-Bred Intl., Inc., Box 4428, Greenville MS 38704.
 Hoy, Daniel, RR 6, Chatham, Ontario Canada O0N7M-05J6.
 Hymowitz, Ted, AW-115 Turner Hall, Univ. of Illinois, 1102 S. Goodwin Ave., Urbana IL 61801.
 Inouye, Jun, Inst. of Trop. Agric., Kyushu Univ., Hakozaki Higashi-Ku, Fukuoka 812, Japan.
 Inst. za Ratarstro I, 45921, Povrtarstro Biblioteka, M Gorkog 30, 21000 Novi Sad, Yugoslavia.
 Inst. za Kukurz, Slobodana Bajica, Box 89, 11080 Zemum, Yugoslavia.
 Jennings, Clark, Pioneer Hi-Bred Intl., Inc., 3261 W. Airline Hwy., Waterloo IA 50703-9610.
 Joshi, Jagmohan, SoyRes. Inst., Dept. Agric., Univ. Maryland Eastern Shore, Princess Anne MD 21853.
 Kenworthy, William, Dept. of Agronomy, Univ. of Maryland, College Park MD 20742.
 Kiang, Yun Tzu, Dept. of Plant Biology, Univ. of New Hampshire, Durham NH 03824.
 Kilen, T.C., USDA-ARS, Delta Branch Exp. Stn., PO Box 196, Stoneville MS 38776.
 Kim, Jin-Key, Dept. Agronomy, College of Agriculture, Chonbuk National University, Chonju 560-756 Korea.

- Kim, Seaok-Dong, Upland Crop Div.(1). Crop Exp. Stn., RDA, Suwon 440-100, Republic of Korea.
- Kim, Yong-Chul, Yeongnam Crop Exp. Stn., RDA, Milyang, Gyeong-nam 627-130, Republic of Korea
- Knapp, A.D., 125 Seed Sci. Bldg., Iowa State Univ., Ames IA 50011.
- Koelling, Paul, Pioneer Hi-Bred Int'l., Inc., 3261 West Airline Hwy., Waterloo IA 50703-9610.
- Konieczny, G., Acad. Agric., Dept. of Genetics and Plant Breeding, 60-625 Poznan, ul. Wojska Polskiego 71 c, Poland.
- Konovsky, J., East-West Seed Co., 1023 S. Adams Suite 1183, Olympia WA 98501.
- Kukuruz, Institut za, Slobodana Bajica I., PO Box 89, 11080 Zemun, Yugoslavia.
- Kwon, Shin Han, Dept. of Agronomy, College of Industry, Kyung-Hee Univ., Seoul 130-701 Korea.
- Kwon, Y.C., Dept. of Agronomy, Geongsang Natl. Univ., Chinju, 660-701, Kyungnam, Korea.
- Lam-Sanchez, Alfredo, Plant Sci. Dept., School of Agric. & Veterinary Sci., State Univ. of Sao Paulo, 14870 Jaboticabal SP. Brazil.
- Larson, Don, Silk Rd. Trading Co., 8619 Reseda Blvd., No. 202, Northridge CA 91324.
- Lee, Hong Suk, Dept. of Agronomy, College of Agriculture, Seoul Natl. Univ., Suweon 44-744 Korea.
- Leffel, Robert, Bldg. 011, HH19, BARC-West, Beltsville MD 20705.
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- Lin, M.S., Dept. of Botany, Chung-Hsing Univ., Taichung, Taiwan 40227.
- Lohnes, David, 2303 S. First #103, Champaign IL 61820
- Ludlow, Jeff, Stewart Seeds Inc., RR 8, Box 227, Greensburg IN 47240.
- Madison, J.T., US Plant, Soil Nutrition Lab., Tower Road, Ithaca NY 14853.
- Maugham, S., Library, Dept. of Agriculture, Baron-Hay Court, South Perth, Western Australia 6150.
- Maw, S., Dept. Botany, Natl. Chun-Hsing Univ., Taichung Taiwan 40227.
- McBroom, Roger L., Northrup King Co., 306 Meadow Dr., Box Z, St. Joseph IL 61873.
- McCall, L.L., FFR Coop, RR 1, Box 78, Bells TN 38006.
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- McMullan, Barbara, RR 2, Box 411, AgriPro BioSciences Inc., Brookston IN 47923.
- Mebrahtu, Tadesse, Box 318, VSU, Petersburg VA 23803.
- Mohamed, A.I., Box 385, VSU, Petersburg VA 23803.
- Murfet, I.C., Pisum Genet. Newsl., Dept. Plant Sci., Univ. Tasmania, GPO Box 252C, Hobart, TAS, 7001 Australia.
- Nawracala, J., Acad. Agric., Dept. of Genetics and Plant Breeding, 60-625 Poznan, ul. Wojska Polskiego 71 c, Poland.
- Nelson, R., Dept. Agronomy, Univ. Illinois, 1102 S. Goodwin Ave., Urbana IL 61801.
- Newhouse, Keith, American Cyanamid, PO Box 400, Princeton NJ 0853-0400.
- Nguyen, John, Ctr. Sahelien de L'ICRISAT, BP 12404, Niamey, Republic of Niger.

- Nickell, Cecil, Dept. of Agronomy, 1102 SA. Goodwin Ave., S308 Turner Hall, Univ. of Illinois, Urbana IL 61801.
- Okabe, Akinori, Tohoku Natl. Agric. Exp. Stn., Kariwano Nishisenboku, Senboku, Akita 019-21 Japan.
- Olivieri, A., Univ. Degli Studi di Udine, Istituto de Produzione Vegetale, 33100 Udine, P.le M. Kolbe 4, Italy.
- Orf, James, Dept. of Agronomy, Iowa State Univ., Ames IA 50011.
- Palmer, Reid, G301 Agronomy, Iowa State Univ., Ames IA 50011.
- Parrini, Paolo, Inst. de Agronomie Gen. E. Colt. Erbacee, 35131 Padova, Italy.
- Parrott, W., Dept. Agronomy, Univ. George, Athens GA 30602.
- Paterson, David, Lakeside Biotechnology Inc., 2201 W. Campbell Park Drive, Chicago IL 60612.
- Pfeiffer, Todd, Dept. of Agronomy, Univ. of Kentucky, Lexington KY 40546-0091.
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- Pracht, James, 1509 W. White Champaign IL 61821.
- Rangappa, M., PO Box 453, VSU, Petersburg VA 23803.
- Rao-Arelli, P., Univ. of Missouri-Columbia, Delta Center, Box 160, Portageville MO 63873.
- Reese, P.F. Jr., Tidewater Agr. Exp. Stn., VPI&SU., PO Box 7219, Suffolk VA 23437-0219.
- Rogers, D.J., Dept. of Primary Industry, Box 23, Kingaroy, Queensland, Australia 4610.
- Rombach, John, United Agriseeds, Box 4011, Champaign IL 61820-4011.
- Rose, I.A., NSW Dept. of Agriculture, Res. Stn. PMB, Myall Vale, Narrabri NSW 2390 Australia.
- Rose, J.L., Heritage Res. Stn., Warwick, Queensland 4370 Australia.
- Saïndon, Gilles, Agric. Canada Res. Stn., Lethbridge AB Canada T1J 4B1.
- Sanbuichi, Takashi, Hokkaido Center Agric., Higashi 6, Kita 15, Naganuma-cho, Yubari-gun, Hokkaido Japan.
- Sapra, V.T., Plant Breeding Dept., PO Box 67, Alabama A&M Univ., Normal AL 35762.
- Sawada, Souhei, Obihiro Univ. Agric. & Vet. Med., Inadacho Obihiro Hokkaido, 080 Japan.
- Sebern, Nancy, DeKalb-Pfizer Genetics, Box 8, Beaman IA 50609.
- Sediyama, T., Dept. Fitotechnia, Universidade Federal de Vicosa, 36570 Vicosa-MG Brazil.
- Seitzer, Joseph, KWS Kleinwanzlebener, Saatzucht AG, Grimsehlstr. 31, D-3352 Einbeck, West Germany.
- Shaker, M.A., 33 Aly Mahmoud St. Apt. 3, Heliopolis, Cairo Egypt.
- Shipe, Emerson R., Dept. of Agron. Soils, Clemson Univ., Clemson SC 29634-0359.
- Shimamoto, Fac. of Agriculture, Hokkaido Univ., Sapporo, Japan.
- Shoemaker, Randy, Dept. of Agronomy, Iowa State Univ., Ames IA 50011.
- Singh, Ram J., Dept. of Agron., Univ. Illinois, 1102 S. Goodwin Ave., Urbana IL 61801.
- Smith, Keith, American Soybean Assn., PO Box 27300, St. Louis MO 63141.
- Soldati, Alberto, ETH Inst. Plant Sci. Eschikon 33, CH-8315 Lindau, Switzerland.
- Soper, John, Pioneer Hi-Bred Intl., Hwy. 71 & 19E, Box 30, Redwood Falls MN 56283.

Specht, James, 309 Keim Hall, Univ. of Nebraska, Lincoln NE 68583-0915.
 Stanton, J.J.Jr., Northrup King, PO Box 340, Hartsville SC 29550.
 St. Martin, S.K., Dept. of Agronomy, OSU, 2021 Coffey Rd., Columbus OH 43210.
 Strachan, Jeffrey, Plant Variety Protection Office, Natl. Agric. Library Bldg,
 Rm 500, 10301 Baltimore Rd., Beltsville MD 20705.
 Takahashi, Ryoji, Dept. of Low Temp. Techn., Hokkaido Natl. Agric. Exp. Stn.,
 Hitsujigoaka, Toyohir-Ku, Sapporo 004 Japan.
 Tinus, Christopher, Asgrow Seed Co., PO Box 210, Marion AR 72364.
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 Umezaki, Teruhisa, Lab. of Crop Sci., Fac. of Agric., Kyushu Univ., Hakozaki,
 Higashi-ku, Fukuoka 812, Japan.
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 Vello, Natal Antonio, Genetica-Esalq-USP, Caixa Postal 83, 13400 Piricaba, SP
 Brazil.
 Voss, D., DeKalb Plant Genetics, 3100 Sycamore Rd., DeKalb IL 60115.
 Vrataric, Marija, BTZNC Agric. Inst., Tenjska Cesta bb. 54000 Osijek
 Yugoslavia.
 Walker, Alan K., Asgrow Seed Co., 1060 E. Hwy. 14, RR 1, Jaynesville WI 53545.
 Walker, Terry, Ill. Foundation Seeds Inc., PO Box 722, Champaign IL 61824-
 0722.
 Webb, D.M., Pioneer Hi-Bred Intl. Inc., 7250 NW 62nd Ave., Johnston IA 50131.
 Williams, Curtis, Hartz Seed Co., PO Box 946, Stuttgart AR 72160.
 Yumoto, Setsuzo, Tokachi Agric. Exp. Stn., Memoru-Cho Kasai-gun, Hokkaido 082
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